



The Review on Spectrophotometric Determination of Synthetic Food Dyes and Lakes

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ABSTRACT

Industrialization of the food systems, including a rise in food processing, has increased the use of food additives such as food dyes, preservatives and sweeteners. Scientists have long been concerned that synthetic food dyes and other additives may contribute to hyperactivity and other disturbed behavior in children. The requirements of a satisfactory analytical method are usually manifold, but certainly selectivity is counted amongst the most important. Hence there has always been interest in procedures that can improve the sensitivity and selectivity of the measurement methods themselves. The purpose of this work is to present a review of some Spectrophotometric analytical methods like Derivative Spectrophotometry, H-Point Standard Addition Method (HPSAM), Cloud Point Extraction Method (CPE) to analyses water soluble (Direct dyes) and water insoluble food dyes (lakes).

Keywords: Derivative spectrophotometry, H-point standard addition method, and cloud point extraction

1. INTRODUCTION

Food dyes play a significant role in food industry as food quality and food flavor are closely associated with color of the food. Industrialization of the food systems, and rise in food processing, has increased the use of food additives such as food dyes, preservatives and sweeteners. Water soluble dyes called direct dyes are used in beverages, bakery, dairy goods, and other products. Water insoluble dyes called lakes are made by combining dyes with salts to make insoluble compounds and they tint by dispersion. Lakes are not oil soluble but are oil dispersible. Lakes are most stable than dyes and are ideal for coloring products containing fats and oils or items lacking sufficient moisture to dissolve dyes. Typical utilization include coated tablets, cake and doughnut mixes, hard candies and chewing gums, lipsticks, soaps, shampoos, talc etc.(FDA,2010) Ref: FDA, 2010, FDA/CFSAN food color facts "Food and Drugs Administration". Food coloring is used both in commercial food production and domestic cooking.

Synthetic food dyes, mostly petroleum derived, are U.S approved for use in foods under the foods, drugs and cosmetics Act of 1938. Natural dyes are unstable and easily undergo degradation during the food processing. In European Union the use of following synthetic food dyes (SFD): Amaranth(E123), Ponceau 4R (E124), Erythrosine (E127), Brilliant Blue (E133), Tartrazine (E102), Sunset Yellow (E110), Eosin, Sudan I , Sudan II, Sudan III, Sudan IV are allowed [1].

Synthetic food dyes gives intense and permanent color to food products [2]. The costs of production process are much lower in comparison with production of natural dyes. These advantages encourage producers to use them, in spite of many resources which confirm their negative influence on human health. The color of food is an integral part of our culture and enjoyment of life. But food dyes can cause serious disorders and diseases like nausea, headache, ulceration, lung cancer, hyperactivity, anemia [3], and also have effect on eye, skin, mucous membrane etc. That's why there is a

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necessity to control the content of these dyes in food. For that purpose many analytical methods have been developed like: Derivative spectrophotometry, H-point standard addition method and Cloud point extraction.

In this article, Derivative spectrophotometry methods, H-point standard addition method and Cloud point extraction have been reviewed for food colorants.

2. DERIVATIVE SPECTROPHOTOMETRY

Derivative Spectrophotometry has found wide application in the analysis of multicomponent samples. This technique is based on the use of derivative spectra resulting from derivatisation of zero order, first order, second order etc. in (Figure 1) of UV-Visible absorption spectra [4-13]. The obtained derivative spectra yield a more characteristics profile in comparison to the parent one as new maxima, minima, and points where derivative spectra crosses the X- axis appear.

The Beer's law, in derivative, form assumes the following form:

$$D^n = d^n A / d \lambda^n = d^n \epsilon c l / d \lambda^n$$

Where D is the value of derivative of n^{th} order at wavelength,

ϵ is the molar absorption coefficient,

is the thickness of absorption layer.

As the additively law is kept, the derivative spectrum of mixture is the sum of derivative spectra of each individual component:

$$D^n_{\text{mix}} = D^n_1 + D^n_2 + \dots \dots \dots D^n_x$$

Where the value of n -order derivative of mixture at analytical wavelength, $D^n_1, D^n_2, \dots, D^n_x$ are the values of n -order derivative at analytical wavelength of $1^{\text{st}}, 2^{\text{nd}}, \dots, x^{\text{th}}$ component of mixture. The features mentioned above allow the determination of several components (x) in a mixture by measuring the amplitude of derivative spectrum of mixture at several (minimum x) wavelengths.

The most characteristic feature of second order derivative is a negative band with the minima at the same wavelength as the maximum on the zero order bands. It also shows two additional positive satellite bands on either side of the main band. The fourth derivative shows a positive band. The presence of strong negative or positive band, with the minimum or maximum at the same wavelength as λ_{max} of the absorbance band, is characteristic of even order derivatives. The number of bands observed is equal to the derivative order plus one ($n+1$). The additional property of derivative spectrophotometry as compared with the classical method is the dependence of derivatisation result on shape of zero order spectra. Signals of analyte which are narrow in basic spectrum undergo amplification, whereas broad even intense zero order signals undergo flattening. In the end derivatisation leads to their zeroing. This property allows eliminating the influence of background and increases selectivity of determination. The greatest advantage of Derivative Spectrophotometry is that, broad bands are suppressed relative to sharp bands to an extent that increases with derivative order.

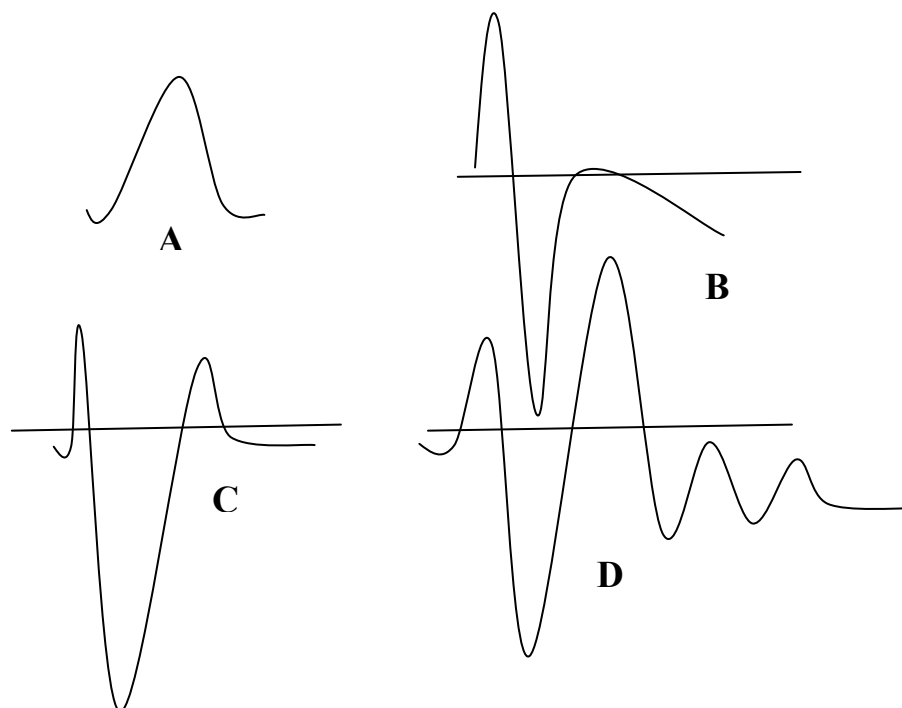


Figure 1. General Derivative Spectra (A) Zero order (B) First order (C) Second order (D) Third order

Table 1: Examples of derivative Spectrophotometric studies of synthetic food dyes.

Food colorants	Sample preparation / Reagent used	Order of derivative spectrophotometry	pH	Wavelength Selection	Concentration range	Detection limit	Application	Reference Number
Carmoisine and Patent Blue	Gelatin desserts and C ₁₈ - cartridge	First	–	465 and 655nm	–	–	Spiked sample	[4]
Tartrazine (TT) and sunset yellow (SY)	Methylene chloride	First	–	429nm (TT) and 485nm (SY)	0.5-10 µg mL ⁻¹ TT and 0.5-12 µg mL ⁻¹ SY	26 and 11 ng mL ⁻¹	Cosmetic products such as : Shampoo, bath gel	[5]
Riboflavin and Sunset yellow (SY)	–	Zero-crossing	4.5	481.7nm and 445.5nm	25.0 µg mL ⁻¹ (Riboflavin) and 40.00 µg mL ⁻¹ (SY)	–	Powder drink samples	[6]
Amaranth, Ponceu4R and Carmoisine	–	First derivative ratio spectra	4.8	522nm (Amaranth), 393nm (Ponceu4R) and 427nm (Carmoisine)	Upto 32 mg L ⁻¹	–	Food samples, drink and cosmetic samples	[7]
Procion yellow HE4R, Procion Red HE7B and Remazol Black5 (RB5)	–	First derivative ratio spectra	–	395nm (HE4R), 659nm (RB5) and 604nm (HE7B)	–	0.180 mg L ⁻¹ (HE4R), 0.317 mg L ⁻¹ (RB5) and 0.0233 mg L ⁻¹ (HE7B)	Deionized water sample	[8]

Coccine, Ponceau6R and Scarlet GN	Aqueous solution	Multivariate data, first and second derivative spectrophotometry	4.5	300-650nm with 0.427nm interval	1.0 -20.0 $\mu\text{g mL}^{-1}$	–	Beverage and candies	[9]
Sunset yellow(SY) and Quinoline yellow (QY)	–	Second–order derivative spectrophotometry and zero crossing derivative spectra	4.5	410nm (QY) and 533.1nm (SY)	–	–	Syrups and tablets	[10]
Tartrazine and Allura red	0.1M HCl	Zero crossing spectra using continuous wavelength transform	–	294.7 nm (Tartrazine) and 555.0 nm (Allura red)	5-30 g mL^{-1} (tartrazine) and 4-24 g mL^{-1} (Allura Red)	–	–	[11]
Tartrazine (TT), Quinoline yellow (QY) and Patent Blue V (P)	–	Second derivative at zero crossing wavelength	4.8	493nm (TT), 463.5nm (QY) and 640nm (P)	20.0 mg L^{-1} (T), 20.0 mg L^{-1} (QY) and 6.4 mg L^{-1} (P)	0.0526 mg L^{-1} (T), 0.0164 mg L^{-1} (QY) and 0.0034 mg L^{-1} (P)	Confectionery stuff, refreshing drink samples	[12]
Amaranth (A), Brilliant Blue(BB), Sunset Yellow(SY), Tartrazine(TT)	Sorbed onto polyurethane foam sodium dodecyl benzene sulfonate	First derivative at zero crossing wavelength	3.0	531.5nm (TT), 578.1nm (A), 520.5nm (SY) and 479.5nm (BB)	1-20 mg L^{-1}	0.057-0.013 mg L^{-1} (T), 0.089-0.002 mg L^{-1} (SY), 0.902-0.043 mg L^{-1} (A), 0.125-0.001 mg L^{-1} (BB)	Gelatine powder(orange, lemon, green apple, raspberry), Juice powder (orange, mango, strawberry)	[13]

In (Table I) Carmoisine and Patent Blue V were simultaneously determined by Yuksel Ozdemir et al [4] in their binary mixtures using first order-derivative spectrophotometry. The method was applied to different gelatin desserts. A sample background correction procedure involving a C₁₈-Cartridge [14] sample preparation step was developed for determination of dyes in gelatin desserts by first order-derivative spectrophotometry. The C₁₈-Cartridge sample preparation step improves recovery in determination of Carmoisine and Patent Blue V in gelatin dessert by first order-derivative spectrophotometry. A Spectrophotometric method for the simultaneous determination of Tartrazine (TT) and Sunset Yellow (SY) in cosmetic products has been developed by Luis Fermin Capitan-Vallvey et al. [5] An extraction process was carried out using Methylene chloride and the coloring matters [15] were measured in the aqueous phase formed, the other components of the sample remaining in the organic phase. A simple and rapid Spectrophotometric method using measurements at zero-crossing wavelength is described by Mahmure Üstün Ögür et al. [6] for resolving binary mixtures of Riboflavin (E-101) and Sunset Yellow (E-110) in a powder drink. The assay procedure for E-110 and E-101 involve the extraction of the colorants from the powder drink with pH 4.5 acetate buffer, appropriate dilution, and measurement of the first order-derivative [16-17] absorbance values. A method for the simultaneous determination of Amaranth, Ponceau 4R and Carmoisine dyes in ternary mixtures with no separation step is proposed by J.J Berzas Nevado et al. [7] This is based on the simultaneous use of the first derivative of ratio spectra and measurements at zero crossing wavelengths. A first order derivative Spectrophotometric method for the simultaneous determination of the three textile dyes, Procion Yellow HE4R, Procion Red HE7B and Remazol Black 5(RB5), has been developed by Vitor C. Almeida et al. [8] The effects of pH, heating and ionic strength of the solution on the absorption spectra of the dye were investigated. The proposed method was applied for the determination of dyes in binary and ternary mixtures of textile effluents [18-19]. Mohammad Reza Oveisi et al [9] analyzed spectrophotometrically a mixture of food colorants, containing Coccine, Ponceau 6R and Scarlet GN simultaneously without prior chemical separation. Sixty mixtures of colorants with three-components were evaluated and the spectrograms were smoothed through the use of seven experimental points [20-22]. These food dyes often used for dyeing foods, drinks, medicine and cosmetics. A very simple spectrophotometric method using measurements at zero-crossing wavelength is described by Mahmure Üstün Özgür et al. [10] for resolving binary mixtures of the food dyes, Sunset Yellow and Quinoline Yellow. Calibration graphs were linear up to 15.0 µg mL⁻¹ of Sunset Yellow and Quinoline Yellow. This method was used for determining synthetic mixtures of these dyes in different ratios and it has successfully been applied to two commercial products without a prior separation step. Resolving binary mixtures of food dyes: Amaranth, Brilliant Blue, Sunset Yellow and Tartrazine, using the first-derivative spectra with measurements at zero-crossing wavelengths have been

described by Eliane C. Vidotti et al. [13] Before the spectrophotometric measurements, the dyes were Sorbed onto polyurethane foam and recovered in sodium dodecyl benzene sulfonate solution. Therefore, matrix complexity was eliminated and simple spectra were obtained. Wavelets transform method has successfully been applied to the analysis of the binary mixtures containing different colorants in commercial food products by Hakan A. Aktas et al. Wavelet transform method [23] is suitable for the quantitative resolution of the mixtures of these colorants and this hybrid approach do not require any separation and extraction step. J.J .Berzas Nevado et al [12] described a spectrophotometric method for resolving ternary mixtures [7] of the food dyes Tartrazine, Quinoline Yellow and Patent Blue V by using the second derivative of the spectra with measurements at zero-crossing wavelengths.

3. H-POINT STANDARD ADDITION METHOD

In 1988, Bosch- Reig et al [24-39] presented a new technique referred to as H-Point Standard Addition Method (HPSAM) for resolving spectra of two analyte with strongly overlapping spectra. This method is based on the dual wavelength spectrophotometry and standard addition method. HPSAM has been applied to remove the blank bias error [40-41] caused by the use of absorbent blank. This method (HPSAM) is highly versatile and can be applied to a number of systems. HPSAM has also been applied for the study of two components called Binary-HPSAM [42] and study of three components called Ternary-HPSAM [43]. The greatest advantage of HPSAM is that it transforms the incorrigible error resulting from the presence of a direct interferent into a constant systematic error and makes it possible to determine the concentration of analyte in the presence of a direct interferent not only the concentration of analyte but that of interferent can also be determined simultaneously. Speciation for the analysis of kinetic data [44] with time as an additional variable, and in equilibrium study in micellar media HPSAM can be used for simultaneous determination of two components in the solution in case of food dyes and some toxic metal ions.

HPSAM Method

HPSAM is a simple two variable chemometric technique. For applying binary HPSAM consider a sample containing an analyte X and an interferent Y. To determine the concentration of analyte X in the presence of interferent Y by binary- HPSAM requires the selection of two wavelengths λ_1 and λ_2 at which the interferent species Y should have the same absorbance. Appropriate wavelengths pairs are selected by the following principles:

- At the two selected wavelengths, the signal of interferent species must remain same, even if the concentration of analyte is changed.
- The analytical signals of the mixture composed from the analyte (X) and the interferent (Y) should be equal to the sum of the individual signals of two species.

- In order to get good accuracy and precision, the slope difference of the straight lines obtained at λ_1 and λ_2 must be as large as possible.

It is possible to select several wavelength pairs where the absorbance is same for the interferent species. But, the wavelength pairs are selected following the criteria to give high values for the difference of the calibration slopes because higher the value for the slope increment the smaller the error for the analyte concentration. After the selection of appropriate wavelength pair (λ_1 and λ_2) known amounts of analyte are successively added to the mixture and the resulting absorbance is measured at the two selected wavelengths and is given by the following equations:

$$A(\lambda_1) = b_0 + b + M_{\lambda_1} C_i \quad (1)$$

$$A(\lambda_2) = A_0 + A' + M_{\lambda_2} C_i \quad (2)$$

Where,

- $A(\lambda_1)$ = analytical signal of the sample measured at λ_1
- $A(\lambda_2)$ = analytical signal of the sample measured at λ_2
- b_0 = the original analytical signal of X at λ_1
- A_0 = the original analytical signal of X at λ_2
- b = analytical signal of Y at λ_1
- A' = analytical signal of Y at λ_2
- M_{λ_1} = slope of the standard addition calibration line at λ_1
- M_{λ_2} = slope of the standard addition calibration line at λ_2
- C_i = added X concentration.

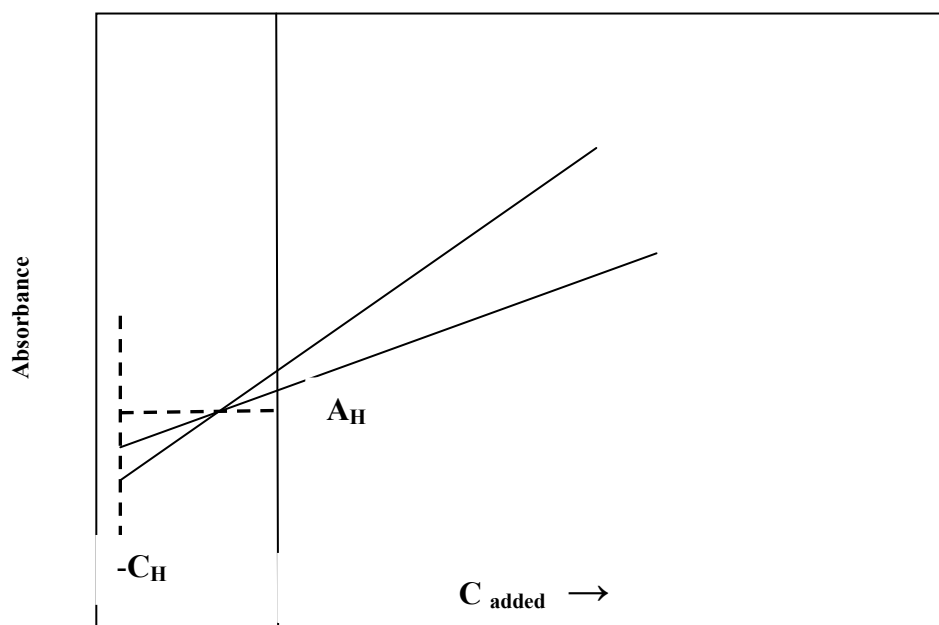


Figure 2: Plot of Absorbance v/s Concentration of analyte added (C_X) by H-Point Standard Addition Method (HPSAM)

With the standard addition method the unknown concentration of analyte can be calculated. Starting from the concentration values of the pure analyte at the intersection of the line with the abscissa, then for the first solution, which contains only the sample and no added analyte, the analytical signal value is the absorbance due to interferent and this absorbance value is constant at the selected wavelengths, all straight lines obtained at different wavelengths at which interferent has the same absorbance, by applying Standard addition method will have a common point. This point is the H-Point, the abscissa being interferent. From this the concentration of interferent can be determined when it is known to be present and when the corresponding calibration graph is also known. The two straight lines obtained at the so-called H-Point ($-C_H, A_H$) (Figure 2).

At H-Point, since $A(\lambda_1) = A(\lambda_2)$, $C_i = -C_H$, so equating Eqs. (1) and (2),

$$b_0 + b + M_{\lambda_1} (-C_H) = A_0 + A' + M_{\lambda_2} (-C_H), \quad (3)$$

$$-C_H = [(A_0 - b_0) + (A' - b)] / (M_{\lambda_1} - M_{\lambda_2}) \quad (4)$$

From Eq. (4), it is concluded that:

$$A' = b$$

- **If the component Y is the known interferent then:**

$$-C_H = (A_0 - b_0) / (M_{\lambda_1} - M_{\lambda_2})$$

$$-C_H = -b_0 / M_{\lambda_1} \quad (5)$$

Where $C_H = C_X$ corresponds to the analyte concentration in the mixture, as $-C_H$ depends only on variables related to the analyte. This is equivalent to:

$$-C_H = -A_0 / M_{\lambda_2} \tag{6}$$

If the value of $-C_H$ is included in equation (1), A_H , the ordinate value of the intersection point, is given as follow:

$$A_H = b_0 + b + M_{\lambda_1} (-C_H), \tag{7}$$

as $b_0 = M_{\lambda_1} C_H$ [Eq. (5)], then

$$A_H = b, \tag{8}$$

And similarly,

$$A_H = A' \tag{9}$$

Hence, A_H value is only related to the signal of the interferent Y at the selected wavelengths. The

concentration of interferent can be determined from its calibration graph. At H-Point, C_H is independent from the concentration of interferent and also A_H is independent from the concentration of analyte.

➤ **if the component Y is the unknown interferent,**

Equation (4) will be tenable as long as the analytical signals due to interferent (b at λ_1 and A' at λ_2) remain equal with the addition of analyte X. Determination of the analyte concentration, X, in the presence of the unknown interferent by generalization H- Point standard addition method (GHPSAM) has also been reported.

Table 2: Applications of some food dyes for H-Point standard addition method (HPSAM)

Food Colorants		pH	Surfactant used	Wavelength pairs	Reaction time / Linear range	References No.
(1)Carmoisine (2)Ponceau 4R	–	4.5	–	490 & 549 nm and 490 & 541 nm	–	[42]
(1)Tartrazine, (2)Sunset yellow (3)Ponceau 4R	–	4.5	–	382.0 & 460.1nm , 366.2 & 446.0nm and 501.7 & 526.0nm	–	[43]

According to (Table II) a new, simple, sensitive and inexpensive H-point standard addition method has been developed by Manna Hajimahmoodi et al [42] for simultaneous determination of binary mixtures of food colorants Carmoisine and Ponceau 4R, which are species with highly overlapped spectra. Absorbance at the two pair of wavelengths, 460 and 549nm or 490 and 541nm, were monitored while adding standard solutions of Carmoisine or Ponceau 4R, respectively. This method has satisfactorily been applied for the determination of Carmoisine and Ponceau 4R in synthetic binary mixtures and several commercial products. Yongnian Ni et al [43] has developed a simple UV spectrophotometric method for resolving ternary mixture of Tartrazine, Sunset Yellow and Ponceau 4R which are species with highly overlapped spectra, by H-Point Standard Addition Method (HPSAM). This method was satisfactorily applied to determination of colorants of Tartrazine, Sunset Yellow and Ponceau 4R in synthetic ternary mixtures and several commercial products such as Candy, Beverages and Food samples.

4. CLOUD POINT EXTRACTION

The cloud point extraction offers a simple, sensitive, inexpensive, and non-polluting and environmentally benign methodology which is alternative to other separation/ pre-concentration technique. Cloud point extraction has been used in several different matrices for pre-concentration of trace amounts of organic and inorganic species determination [44-51]. Cloud point extraction offers many advantages over traditional liquid-liquid extraction. Compared with traditional liquid-liquid extraction, it uses water and non toxic surfactants, avoids the use of large amounts of toxic, carcinogenic and flammable organic solvents. It agrees with green chemistry principles. It can lead to higher recovery efficiency and a large pre-concentration factor because the presence of the surfactant can minimize losses of analyte due to their adsorption onto the container. The cloud point extraction method gives very low limit of detection (LOD), good Standard deviations(SD'S); more eco-friendly .It is a combination of sensitivity, selectivity, simplicity and relatively short time of analysis.

The three important parameters in cloud point extraction are: -

- Surfactant
- Incubation Time
- Equilibration temperature

Surfactant i.e. surface active product [52], they form a unique class of chemical compounds. Soaps fatty acids salts containing at least eight carbon atoms and detergents all are surfactants. Gemini surfactant, coined by Menger [53] are dimeric surfactant i.e. surfactant molecules have two hydrophilic group and two tails per surfactant molecules [54]. In aqueous solution dilute concentration of surfactant act as normal electrolytes, but at higher concentrations, they form organized aggregates of large numbers of molecules called Micelles, in which the lipophilic parts of surfactants associate in the interior of the aggregate leaving hydrophilic parts to face the aqueous medium. The formation of micelles in aqueous solution is a compromise between the tendencies for alkyl chains to avoid energetically unfavorable contacts with water and desired for the polar parts to maintain contacts with aqueous medium. Aqueous solutions of most non ionic

and zwitterionic surfactants, when heated or cooled, become cloudy over a narrow temperature range to the diminished solubility of the amphiphile in water [55-57]. This critical temperature is called the "Cloud point". Above the cloud point, the solution separates into two isotopic liquid phases :- One very small in volume, the surfactant-rich phase (coarsevate) and the other, the dilute aqueous phase, in which the surfactant concentration is close to its critical micelle concentration.

In the cloud point extraction it is important to choose the correct Surfactant. If the cloud point temperature of the surfactant is too low, the phase separation is easy but the concentration efficiency is low because of the low solubility in aqueous solution [58].

The effects of Incubation time and Equilibration temperature in cloud point extraction, they are necessary to complete extraction and to achieve easy phase separation.

The cloud point extraction process (CPEP) technique has been used for extraction pre-concentration [59], separation and purification of food colorants, biomaterials, organic compounds, and metallic species.

Table 3. Examples of Spectrophotometric conditions for Cloud point extraction method

Name of dyes	Reagent used	Surfactant	pH	Equilibration temperature	Incubation time	Detection limit	Wavelength	Application's	Reference No.
Amaranth	Tetra-Butyl ammonium hydrogen sulfate, Na ₂ SO ₄ (1.0M)	Triton X-100	4.5	80 ⁰	30 min	13.0	518 nm	Beverage, Jelly 1, Jelly 2, Jelly 3	[60]
Brilliant Green	Benzoic acid	Triton X-100	3.5	45 ⁰	10 min	0.015	628 nm	In water sample: River water, In fish sample: fish farming water	[61]
Sudan (I-IV)	Na ₂ CO ₃	Triton X-100	—	70 ⁰	30 min	2-4	500 nm	Chilli powder	[62]
Malachite Green	Na ₂ SO ₄	Triton X-100	2.2	60 ⁰	20 min	0.87		In fish water sample	[63]
Rhodamine - B	NaCl	Triton X-100	3.5	—	30 min	1.3	563 nm	Soft paste, hand washing liquid soap,	[64]
Congo Red Eosin Chrysoidine	—	Triton X-100, Triton X-114	—	343K, 348K, 353K, 358K, 363K, 368K	—	—	499 nm 517 nm 457 nm	—	[65]
Orange (II) azo dye	KCl	Triton X-100, CTAB	3.0	70 ⁰	25 min	0.67	484 nm	Chewing gum, Sweet samples	[66]

In (Table III) Cloud point extraction methodology has successfully been employed by N.Pourreza et al [60] for preconcentration of trace amounts of Amaranth prior to its determination by spectrophotometry. The method is based on the extraction of amaranth as ion pair with tetrabutylammonium ion from aqueous solution [67] using Triton X-100 as non-ionic surfactant. The extracted surfactant rich phase was diluted with ethanol and its absorbance value was measured at 518 nm. An optimum set of surfactant concentration, pH, equilibration temperature and time, tetrabutylammonium hydrogen sulfate and salt concentration were obtained. A micelle-mediated phase separation method for preconcentration of Brilliant Green (BG) by using spectrophotometer has been developed by Hossein Tavallali et al. [61] The method is based on the cloud point extraction of BG at pH 3.5 mediated by micelles of nonionic surfactant Triton X-

100. The effect of different variables were evaluated and optimized to enhance the sensitivity and extraction efficiency of the proposed method. The method was applied to determine BG in different fish farming and water samples. Wei Liu et al [62] determined Sudan (I-IV) dyes by CPE-HPLC (UV) method using non-ionic surfactant Triton X-100. The separation and determination of Sudan dyes was then carried out in the system with isocratic elution, and the detector was set at 500 nm. The various parameters and variables that affect the extraction were investigated. Xubiao Luo et al [63] employed a high-sensitivity off-line coupled with on-line preconcentration method, cloud-point extraction (CPE) and sweeping-MEKC, for the analysis of Malachite green [68]. The optimal conditions were 250 g/L of Triton X-100, 10% of Na₂SO₄ (w/v), heat-assisted at 60^oc for 20min. A new micelle-mediated cloud point extraction method is described for sensitive and selective determination of trace amounts of Rhodamine B by N.Pourreza et al [64]. The method is based on the cloud point extraction of Rhodamine B from aqueous solution using Triton X-100 in acidic media. The effects of different operating parameters such as concentration of surfactant and salt, temperature and pH on the cloud point extraction of Rhodamine B were studied in detail. The method was applied for the determination of Rhodamine B in soft pastel, hand washing liquid soap, matches tip and textile dyes mixtures samples. M.K.Purkait et al [65] have studied three different dyes i.e. Congo red, Eosin and Chrysoidine. Iso-octyl phenoxy polyethoxy ethanol and octyl phenol poly ethylene glycol ether were used as non-ionic surfactant. The effects of different operating conditions of temperature and concentration of surfactant and dyes on various thermodynamic parameters such as change in Gibbs free energy (ΔG^0), change in enthalpy (ΔH^0) and change in entropy (ΔS^0) were studied in detail. The values of ΔG^0 increase with temperature but decreases with the concentrations of surfactant and dye. The values of ΔH^0 and ΔS^0 increase with surfactant concentration but decrease with dye concentration. A method based on the extraction of Orange II from aqueous solution using mixed micelles of non-ionic surfactant, Triton X-100 and cationic surfactant cetyltrimethyl ammonium bromide in acidic media has been developed by Pourreza N et al [66].

Orange II is one of the azo-dyes, which is widely used as coloring agents in a variety of products, such as textile, paper, foodstuffs, hair dye and leather.

5. CONCLUSION

Different spectrophotometric techniques such as derivative spectrophotometry, H-point standard addition method and Cloud point extraction method have been explained for the analysis of synthetic food dyes. Each of the described methods can be used to analyse synthetic food dyes successfully. For qualitative and quantitative determination of synthetic food dyes, these are the best solution. It enables obtaining satisfactory results in short time. Choice of spectrophotometric technique is dependent on the method of sample preparation. For Derivative spectrophotometry and H-point standard addition method sample must be carefully purified, that assures satisfactory simultaneous determination possible.

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