Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1153981

57 (2016) 25822–25829
November

Taylor & Francis
Taylor & Francis Group

Solid-phase extraction of some food dyes on sea sponge column and determination by UV–vis spectrophotometer

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Received 21 May 2015; Accepted 9 February 2016

ABSTRACT

Preconcentration methods are widely used before the determination of analytes, which have low concentration values in the samples. One group of these analytes which need to be preconcentrated before determination is food dyes. This work was aimed to develop a new method to determine Ponceau 4R and Sudan Orange G dyes by using a column filled with sea sponge, as solid-phase. Sea sponge is firstly used in this work for the preconcentration of the food dyes. The method is applied to various food samples after optimizing analytical parameters such as HCl concentration, eluent type, sample volume, flow rates of solutions, and matrix effects. The detection limits based on three times the standard deviations for the blanks were found in the range of 0.002 μg/L and 1.9×10^{-4} μg/L for Ponceau 4R and Sudan Orange G dyes, respectively. Calibration standards were prepared in the ranges of 8.5×10^{-7}–8.5×10^{-5} mol/L and 2.34×10^{-5}–2.34×10^{-3} mol/L for Ponceau 4R and Sudan Orange G dyes, respectively, and the correlation coefficient was 0.999.

Keywords: Solid-phase extraction; Sea sponge; Ponceau 4R; Sudan Orange G

1. Introduction

Analytical chemistry deals with the determination of analytes in various media. Food dyes are important food additives and color is the first sensory parameter by which food quality and flavor are judged. As with many other food additives, the analytical control of these dyes is of considerable importance in food industry because of their toxic and carcinogenic potential [1]. Food additives are commonly used in processed foodstuffs to improve appearance, flavor, taste, color, texture, nutritive value, and conservation [2]. Monitoring the levels of dyes in high consumption products such as beverages becomes therefore of paramount importance [3]. Ponceau 4R (E124) is classified in azo dye group and is one of the dyes which gives red color to food [4]. The water solubility of Ponceau 4R is 30 g/100 mL at 25°C and because of this waste management of this dye is also important [5].

Sudan Orange G is also a member of the azo dyes group and because of its low cost and wide availability as the other Sudan dyes, it is used as food colorants but due to their carcinogenicity Sudan dyes are banned as colorants in food in most countries, including in the EU [6].

Many analytical methods have been used for the determination of food dyes, including spectrophotometry

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[1,7,8], HPLC [9–11] and polarography [12–14]. It is important to separate the dyes from the matrix components and preconcentrate before their determinations. Solid-phase extraction [15–18], cloud point extraction [19,20], and dispersive liquid–liquid microextraction [21,22] methods are used for the separation and preconcentration of the food dyes.

These two dyes, Ponceau 4R and Sudan Orange G, are chosen as analytes for this work because Sudan Orange G is a synthetically produced azo dye. Degradation products of azo dyes are considered to be carcinogens and teratogens. Due to this fact, the EU does not permit the use of these colors as food additives. However, in some countries, these dyes are still occasionally used in order to intensify the color of bell pepper and chili powder. Sudan Orange G and Ponceau 4R dyes are selected for determination because Sudan Orange G is categorized as carcinogens according to International Agency for Research on Cancer (IARC) and its use is illegal [23], and Ponceau 4R is a food dye but there are limits for its use.

Sea sponge, which is sold at markets as commercial goods, was firstly used as solid-phase in this work for the determination of food dyes. The kind of the sponge used in this work is demospongiae which has siliceous spicules and/or fibrous skeleton [24]. It is environmentally friendly and economic to use as solid-phase in a column. It was also previously applied as solid-phase by our group for the preconcentration of some metal ions before their determination by flame atomic absorption spectrometry [24]. The results of our previous work showed that sea sponge is a good adsorbent and this inspired us to use it for the preconcentration of the food dyes. Determination of Ponceau 4R in food samples is important because it is considered carcinogenic in some countries, including the US, Norway, and Finland, and it is currently listed as a banned substance by the US Food and Drug Administration [25]. In Turkey, use of Ponceau 4R in jams, marmalades, and jellies is limited to maximum 100 mg/kg or mg/L, and in candies and beverages, it is limited to maximum 50 mg/kg or 50 mg/L [26].

Ponceau 4R can cause adverse health effects, such as reproductive toxicity, neurobehavioral effects, mutagenic action, and potential carcinogenicity when consumed in excess [27]. The ADI value for Ponceau 4R is 0–4.0 mg/kg based on the Food and Agriculture Organization and World Health Organization recommendations [28]. Any level of Sudan dyes is considered to be unsafe for human consumption, and thus the application of such dyes has been prohibited in many countries. However, they are illegally used as food adulterants, particularly in red chili powder and in chili containing foods such as curry, frozen meats, and spice mixes because of their intense red-orange color and low price [29,30]. Sudan dyes are thought to be carcinogenic, yet have been determined as a contaminant in chili powder [31]. Use of Sudan dyes as additives, at any level, in food products destined for human consumption is banned in many countries [32].

The aim of the present study was to develop a rapid, sensitive, and accurate spectrophotometric method for the determination of commercially available Ponceau 4R and Sudan Orange G dyes, by using sea sponge as adsorbent for dye preconcentration for the first time.

2. Methods

2.1. Reagents and solutions

Analytical reagent grade chemicals were used in this work without further purification. Standard solutions of the dyes were prepared daily in water into 100 mL calibrated flasks. High purity compounds were used for the preparation of the stock solutions of diverse ions and dyes. Buffer solution of pH 4.5 was prepared from acetate buffer solution (CH_3COO^-/CH_3COOH) by mixing of appropriate volumes of 1 mol/L acetic acid and 1 mol/L sodium acetate solutions.

2.2. Instrument

A Hitachi-150-20 spectrophotometer (Hitachi High-Technologies, Japan) with a 10 mm quartz cell was used to measure the absorbance of Ponceau 4R (Sigma-Aldrich Co. (St. Louis, USA)) and Sudan Orange G (Sigma-Aldrich Co. (St. Louis, USA)) dyes at 510.2 nm and 383.2 nm, respectively. A pH meter, Nel pH-900 (Nel Company, Ankara, Turkey) model glass-electrode, was employed for measuring pH values in the aqueous phase. A Human model RO 180 (Human Corp., Seoul, Korea) was used for water purification, resulting water with a conductivity of 1 S/cm.

2.3. Solid-phase extraction procedure

Sea sponge column was prepared by using a glass column (10 cm length and 1.0 cm diameter) with a fritted glass and a stopcock. The column was packed with 500 mg of sea sponge. The packed sea sponge was washed successively with methanol, water, 1 mol L^{-1} HNO_3 in acetone, water, 1 mol L^{-1} NaOH, water, acetone and water to remove organic and inorganic contaminants.

Before applying the method to real samples it was tested on model solutions. Model solutions were prepared by adding 200 µg of Ponceau 4R and 400 µg of Sudan Orange G in a 50 mL beaker and the HCl concentration of the model solutions were 0.02 M. Firstly 0.02 M HCl solution was passed from the column as conditioning solution, then the model solution was passed from the sea sponge column at a flow rate of 3 mL/min. After passing of the model solution was completed, 10 mL of pH 4.5 buffer solution was passed from the column as eluent solution at a flow rate of 3.0 mL/min. The number of replicates for the test workings was three. The levels of Ponceau 4R and Sudan Orange G dyes were determined by UV–vis spectrophotometer at 510.2 and 383.2 nm respectively.

2.4. Applications

Various drink powders and candies sold in Nevşehir and Kayseri-Turkey markets were used for the determination of Ponceau 4R, and tomatoes paste and dried pepper powders sold in Kayseri-Turkey markets were used for the determination of Sudan Orange G. Ponceau 4R containing samples were chosen according to their labels and no other dyes were labeled on the packages. Drink powder and candy samples were tared and solved in water and centrifugated to separate the dissolved material; the solutions were taken and the preconcentration method was applied. For the liquid drink sample, 1 mL of the sample was taken directly and the preconcentration method was applied. About 1 g of each pepper sample was tared and dissolved in water and diluted to 50 mL with water and then filtered with cellulose nitrate membrane filter (Osmonics, Westborough, MA) and the preconcentration procedure was applied to 5 mL of these filtered samples.

3. Results and discussions

In order to obtain quantitative recoveries of Ponceau 4R and Sudan Orange G dyes on sea sponge column, the enrichment/separation procedure was optimized for various analytical parameters such as HCl concentration, sample volume, sample and eluent flow rates, matrix effects, etc.

3.1. Influences of HCl concentration

The effect of HCl concentration on the recovery of Ponceau 4R and Sudan Orange G was investigated by using model solutions in the range of 0.01–0.5 M HCl. The highest preconcentration factors were gained for both dyes when 0.02 M HCl was used; according to the results shown in Fig. 1, 0.0.2 M HCl concentration is selected for the further experiments.

3.2. Eluent type and volume

Various eluting solutions at various concentrations were used for desorption of the retained dyes from the sea sponge column. The flow rates were controlled by a stopcock of the column and for higher flow rates vacuum are applied. The results are given in Table 1. Ponceau 4R and Sudan Orange G dyes were quantitatively recovered from the sea sponge column with 10 mL of pH 4.5 acetate buffer solution and it was used as eluent for further studies.

3.3. Flow rates

Flow rates of the sample solution and eluent solution passing the column is another important parameter. To find the optimum flow rates of sample and eluent solutions on the recovery of dyes, sample solution and eluent flow rates were investigated in the range of 1.0–10.0 mL/min while other conditions were kept constant. The flow rates were controlled by a stopcock of the column and for higher flow rates, vacuum was applied. Results are shown in Figs. 2 and 3 for sample and eluent solutions, respectively.

As seen from the results shown in Fig. 2, Ponceau 4R recoveries were quantitative in the whole range where Sudan Orange G recoveries were quantitative in the range of 3.0–10.0 mL/min. It is clear from Fig. 3 that both dyes were quantitatively recovered in the flow rate range of 1.0–8.0 mL/min.

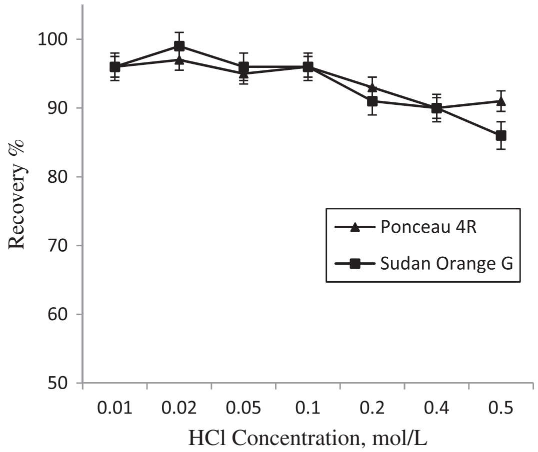

Fig. 1. Effect of HCl concentration on the recovery of Sudan Orange G and Ponceau 4R dyes ($N = 3$).

Table 1
Effect of eluent type and volume on the recovery of Ponceau 4R ve Sudan Orange G dyes (*N* = 3)

		Recovery (%)	
Eluent type	Eluent volume (mL)	Ponceau 4R	Sudan Orange G
Acetone	10	9 ± 1	52 ± 7
1 M NaCl	10	4 ± 1	11 ± 1
pH 6 (phosphate buffer)	10	24 ± 4	6 ± 1
1 M acetic acid	10	4 ± 1	14 ± 3
Ethyl alcohol	10	22 ± 1	53 ± 3
1 M NaOH	10	95 ± 0	95 ± 7
1 M NH_3	10	89 ± 1	87 ± 2
pH 4, 5 (acetate buffer)	10	100 ± 0	99 ± 1
1 M NH_3	5	2 ± 0	4±1
1 M NaOH	5	76 ± 2	98 ± 0
pH 4, 5 (acetate buffer)	5	50 ± 1	81 ± 4
2 M NH_3	5	108 ± 1	91 ± 1

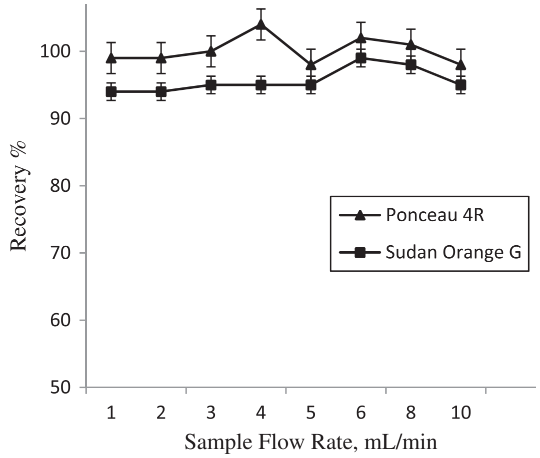

Fig. 2. Effect of sample flow rate on the recovery of Sudan Orange G and Ponceau 4R dyes (*N* = 3).

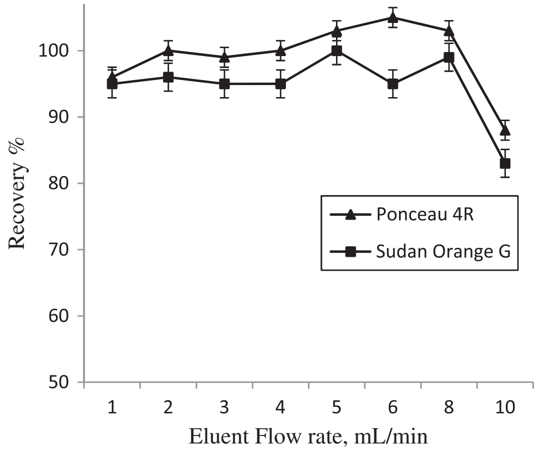

Fig. 3. Effect of eluent flow rate on the recovery of Sudan Orange G and Ponceau 4R dyes (*N* = 3).

3.4. Effect of sample volume

The effects of sample volume on the recoveries of Ponceau 4R and Sudan Orange G dyes on sea sponge column were investigated in the sample volume range of 25–1,000 mL by preparing model solution containing 200 µg of Ponceau 4R and 400 µg of Sudan Orange G. The results in Fig. 4 show that Sudan Orange G was only recovered quantitatively at 25 mL and recovery of Ponceau 4R was quantitative in the whole range.

3.5. Influences of matrix on the recoveries

The matrix effects are a big problem in the solid-phase extraction of analyzes at trace level [33–36]. The effects of common ions and some food dyes on the recoveries of Ponceau 4R and Sudan Orange G was also investigated. For this purpose the proposed preconcentration method was applied to model solutions which contain various ions and food dyes. The results for the effects of ions and dyes are given in Tables 2 and 3, respectively. It is clear from Table 2 that the method is applicable for Ponceau 4R and Sudan Orange G without interference of the investigated ions. According to the results given in Table 3, there is interference only between Ponceau 4R and Amaranth dyes because Amaranth dye has a maximum absorbance at 520 nm while Ponceau 4R has a maximum at 510 nm [37].

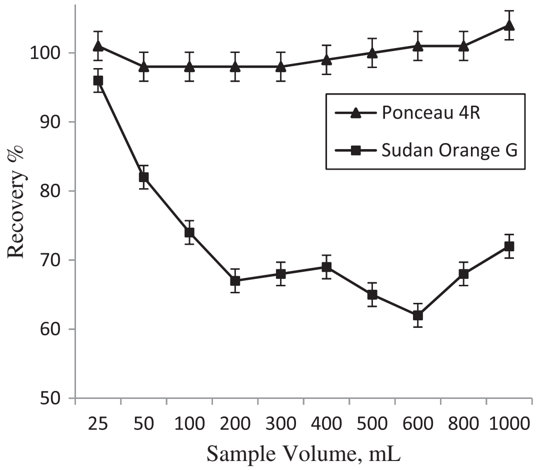

Fig. 4. Effect of sample volume on the recovery of Sudan Orange G and Ponceau 4R dyes ($N = 3$).

3.6. Effects of amount of sea sponge

In solid-phase extraction methods, the amount of the solid-phase is also an important parameter that affects the recovery results. In this part of the experiment the effect of sea sponge amount was investigated in the range of 0.1–1 g. It can be seen from Fig. 5 that quantitative recoveries for Ponceau 4R and Sudan Orange G dyes were possible in the sea sponge amount range of 0.4–0.7 g.

3.7. Analytical performance of the presented method

For the estimation of the accuracy of the presented method, different amounts of the Ponceau 4R were spiked in the range of 0–40 µg in a Rosehip flavored drink powder purchased from a market in Nevsehir-Turkey, and the presented method given in Experiment was applied. It can be said that a good agreement was obtained between the added and

Table 2
Matrix effect on the recovery of Ponceau 4R and Sudan Orange G dyes ($N = 3$)

			Recovery (%)	
Ion	Concentration (mg/L)	Added As	Ponceau 4R	Sudan Orange G
Mg^{2+}	500	$Mg(NO_3)_2$	99 ± 2	95 ± 5
K^+	500	KCl	97 ± 2	95 ± 3
Ca^{2+}	1,000	$CaCl_2$	96 ± 0	95 ± 1
NO_3^-	2,500	$NaNO_3$	97 ± 1	95 ± 2
SO_4^{2-}	1,000	Na_2SO_4	97 ± 4	95 ± 2
PO_4^{3-}	2,500	$NaH_2PO_4{\cdot}2H_2O$	97 ± 2	96 ± 1
CO_3^{2-}	100	Na_2CO_3	96 ± 1	96 ± 3
Co^{2+}	5	$Co(NO_3)_2{\cdot}6H_2O$	95 ± 1	95 ± 3
Cu^{2+}	5	$Cu(NO_3)_2{\cdot}3H_2O$	97 ± 1	95 ± 3
Fe^{3+}	5	$Fe(NO_3)_3{\cdot}9H_2O$	96 ± 1	96 ± 3
Cr^{3+}	5	$Cr(NO_3)_3{\cdot}9H_2O$	99 ± 2	97 ± 3
Pb^{2+}	20	$Pb(NO_3)_2$	100 ± 1	95 ± 3

Table 3
Effects of some other food dyes on the recovery of Ponceau 4R and Sudan Orange G ($N = 3$)

Recovery (%)			
Dyes	Concentration (mg/L)	Ponceau 4R (510.2 m)	Sudan Orange G (383.2 nm)
Chocolate Brown	1	107 ± 3	97 ± 1
Brilliant Blue	1	93 ± 2	95 ± 1
Tartrazine	0.5	95 ± 3	102 ± 4
Sunset Yellow	0.5	98 ± 3	99 ± 1
Patent Blue V	1	97 ± 2	96 ± 3

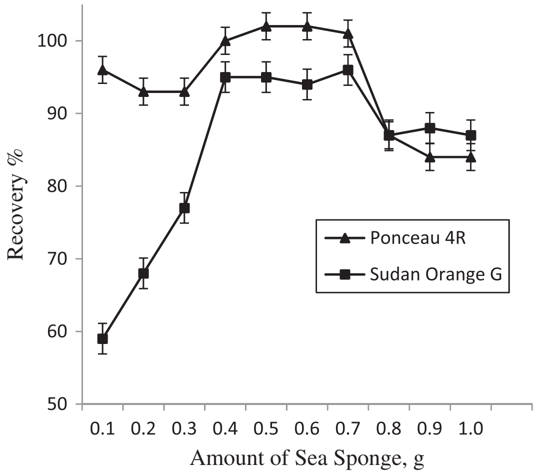

Fig. 5. Effect of sea sponge amount on the recovery of Ponceau 4R and Sudan Orange G dyes ($N = 3$).

Table 4
Tests of addition/recovery for the application of the method on a drink powder sample containing Ponceau 4R ($N = 3$)

Analyte	Added (μg)	Found (μg)	Recovery (%)
Ponceau 4R	0	12 ± 1	–
	10	21 ± 0	98 ± 0
	20	31 ± 0.3	97 ± 1
	40	50 ± 1	95 ± 3

measured dye amounts according to the results shown in Table 4. Table 5 shows the results of Sudan Orange G spike and recovery experiments in a red pepper powder sample in the range of 0–20 μg. The values were quantitative and it means that the presented method can be applied for the separation–preconcentration of these dyes in real samples similar to which we investigated. The detection limits, defined as the concentration equivalent to three times the standard deviation ($N = 15$) of the reagent blank were found as: 0.002 μg/L for Ponceau 4R and 1.9×10^{-4} μg/L for Sudan Orange G.

Table 5
Tests of addition/recovery for the application of the method on a red pepper sample containing Sudan Orange G ($N = 3$)

Analyte	Added (μg)	Found (μg)	Recovery (%)
Sudan Orange G	0	7 ± 0.2	–
	5	12 ± 0.2	96 ± 3
	10	18 ± 1	98 ± 5
	20	27 ± 1	99 ± 7

Table 6
Analysis of Ponceau 4R containing samples ($N = 3$)

Solid samples	Concentration (μg/g)
Peach flavored drink powder	131 ± 8
Fruit flavored mint candy	7 ± 2
Flavored rock candy	33 ± 4
Rosehip flavored drink powder	309 ± 16
Liquid samples	Concentration (μg/mL)
Fruit flavored soft drink	23 ± 1

Table 7
Analysis of Sudan Orange G containing samples ($N = 3$)

Samples	Concentration (μg/g)
Tomatoes paste	28 ± 1
Pepper 1	155 ± 14
Pepper 2	136 ± 6
Pepper 3	94 ± 4

3.8. Analyzing real samples by the presented method

The proposed method was applied to determine the amounts of Ponceau 4R in various drink powder, soft drink, and candy samples collected from Nevşehir and Kayseri-Turkey, and to the determination of Sudan Orange G in tomatoes paste and pepper samples collected from Kayseri-Turkey. The results of Ponceau 4R and Sudan Orange G in the analyzed samples are given in Tables 6 and 7, respectively.

4. Conclusion

In this study, an easy, rapid, and inexpensive method for the preconcentration and separation of Ponceau 4R and Sudan Orange G in some food samples with acceptable accuracy and precision is presented. Matrix components were easily eliminated by the presented method. All data proved that the established method could be effectively applied to the analysis of trace Ponceau 4R and Sudan Orange G in some food and drink samples. The results show that tomato paste and pepper samples contain Sudan Orange G despite being banned and two of the drink powder samples contain Ponceau 4R higher than the allowed limits.

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