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Solid-phase extraction and spectroscopic determination of synthetic dyes Sunset Yellow (E110) and Ponceau 4R (E124) in foodstuffs using alumina modified with polyhexamethylene guanidine

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Alumina, modified with polyhexamethylene guanidine (Al_2O_3–PHMG), was suggested for simultaneous preconcentration and determination of food dyes using diffuse reflectance spectroscopy. Adsorption of synthetic dyes the Sunset Yellow (E110) and Ponceau 4R (E124) was studied; Al_2O_3–PHMG adsorbent extracted dyes in a wide range of acidity at pH 1–8. Synthetic dyes were quantitatively extracted at pH 1-2 and 5-6. The best adsorption capacity was observed at pH 1–2 and was 95 and 85 μmol g^{-1} for Sunset Yellow and Ponceau 4R, respectively. The maxima of bands in the diffuse reflectance spectra were at 470 nm (Sunset Yellow) and 510 nm (Ponceau 4R). An increase in the concentration of dyes on the adsorbent surface led to an increase in the intensity of the band in the diffuse reflectance spectra; this was used for their adsorption-spectroscopic determination. Limit of detection, calculated by the 3*s*-criterion, was 5 μg L^{-1} for Sunset Yellow and 7 μg L^{-1} for Ponceau 4R. The analytical range of dye concentrations was 0.02–10.0 mg L^{-1} for Sunset Yellow and 0.02–11.0 mg L^{-1} for Ponceau 4R. The relative standard deviation did not exceed 0.09 (*n*=10). The proposed method was tested for determination of Sunset Yellow and Ponceau 4R in soft beverages and jelly.

Keywords: Adsorption; Modified alumina; Synthetic food dye; Sunset Yellow; Ponceau 4R; Diffuse reflectance spectroscopy

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Сорбционное концентрирование и спектроскопическое определение синтетических красителей Желтого солнечного заката (E110) и Понсо 4R (E124) в пищевых продуктах с использованием оксида алюминия, модифицированного полигексаметиленгуанидином

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Для концентрирования и определения пищевых красителей методом спектроскопии диффузного отражения предложен оксид алюминия, модифицированный полигексаметиленгуанидином (Al_2O_3–ПГМГ). Изучены условия сорбционного извлечения синтетических красителей Желтый солнечный закат - ЖСЗ (Е110) и Понсо 4R - П4R (Е124); оксид алюминия, модифицированный полигексаметиленгуанидином, извлекает красители в широком диапазоне pH 1–8. Количественное извлечение синтетических красителей наблюдается при pH 1–2 и 5–6. Наибольшая сорбционная емкость достигается при pH 1–2 и составляет 95 и 85 мкмоль/г для красителей ЖСЗ и П4R соответственно. Спектры диффузного отражения представляют собой широкие полосы с максимумами при 470 нм (ЖСЗ) и 510 нм (П4R). С увеличением концентрации красителей на поверхности сорбента увеличиваются и интенсивности полос в спектрах диффузного отражения, что использовано для их сорбционно-спектроскопического определения. Предел обнаружения, рассчитанный по 3*s*-критерию, составил 5 мкг/л для ЖСЗ и 7 мкг/л для П4R. Диапазон определяемых содержаний 0,02–10,0 мг/л для ЖСЗ и 0,02–11,0 мг/л для П4R. Относительное стандартное отклонение не превышает 0,09 (n = 10). Предложенный метод апробирован при определении красителей Желтый солнечный закат и Понсо 4R в безалкогольных напитках и желе.

Ключевые слова: адсорбция; модифицированный оксид алюминия; синтетический пищевой краситель; Желтый солнечный закат; Понсо 4R; спектроскопия диффузного отражения

INTRODUCTION

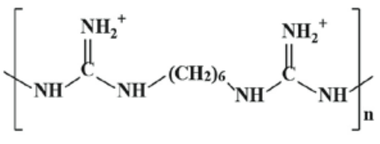
Modern food production is impossible without the use of various food additives. One of the most common additives is synthetic food dyes. Foot dyes are most often added to products such as beverages (soft drinks, low-alcohol drinks, liquor, wine, cognac) and confectionery (candies, pastilles, marmalade).

The need to control the content of synthetic dyes is associated with their negative effect on the human body; almost all of them are harmful and have varying degree of toxicity [1]. Therefore, their content in food products is strictly standardized and requires continuous monitoring. In addition, when discharging wastewater from food processing enterprises, the dyes also enter the environment. High resistance of synthetic food dyes to photo- and biodegradation in the nature leads to their accumulation in the waters. The negative impact of synthetic food dyes on living organisms, especially humans, sets the task of developing simple and express methods for their determination.

Spectrophotometry [2–4], chromatography [5–8], voltammetry [9–10], and capillary electrophoresis [11–13] are used to determine synthetic food dyes.

Often, adsorption preconcentration is used to separate synthetic food dyes from each other and from the matrix components. Various materials of organic and inorganic nature are used as adsorbents. Both biosorbents (pine wood biomass and Arabica coffee residues [14], activated carbon obtained from oak bark [15], Luffa sponge, modified Luffa and activated carbon from Luffa sponge [16], chemically modified brown algae *Sargassum glaucescens* [17]) and synthetic resins (Amberlite XAD-1180 and Amberlite XAD-16 [18], Sepabeads SP 70 [19]) are widely used as organic adsorbents. Modified and unmodified inorganic oxides, silica chemically modified with C16, Diasorb-130-C16, KSK-G silica, γ-Al_2O_3 [20-21], nanoparticles of γ-Al_2O_3 modified with polyacrylamide [22] are the most commonly used among inorganic adsorbents.

Previously, we used aluminum oxide modified with polyhexamethylene guanidine for pre-concentration of the synthetic food dye Fast Green FCF [23]. The amino groups of the modifier (Scheme 1) provide a positively charged adsorbent surface over a wide pH range. This adsorbent extracts organic reagents containing negatively charged sulfo groups, including



Polyhexamethylene guanidine

Sunset Yellow

Ponceau 4R

Scheme 1. Structural formulas of modifier and food dyes water-soluble synthetic dyes such as Sunset Yellow and Ponceau 4R, due to electrostatic interactions.

In this paper, the method of adsorption-spectroscopic determination of the dyes Sunset Yellow (SY) and Ponceau 4R (P4R) using alumina modified with polyhexamethylene guanidine has been proposed.

EXPERIMENTAL

Instrumentation. The UV-Vis spectra and optical density of solutions were registered using Cary 100 spectrometer (Varian, Australia). Diffuse reflectance spectra (DRS) of adsorbents in the range of 380–720 nm were recorded with Pulsar spectrophotocolorimeter (NPO Khimavtomatika, Russia). The spectra are presented in *F*(*R*) – wavelength coordinates, where *F*(*R*) is Kubelka–Munk function, $F(R) = (1 - R)^2/2 \cdot R$ and *R* is the diffuse reflectance coefficient.

The solutions' pH value was measured using a SevenMulti ion meter (Mettler-Toledo, Switzerland). Deionized water with a specific resistance of 18.3 MΩ cm^{-1} was obtained using an E-pure D4642-33 (Thermo Scientific Barnstead & trade, USA) installation.

Thermal analysis was performed by the simultaneous STA 449 C Jupiter thermal analyzer (Netzsch, Germany). The sample was heated in a platinum crucible from 30 to 760 °C at a rate of 10 °C min^{-1}, and air was blown at a rate of 50 mL min^{-1}.

Reagents. The stock solutions of the dyes Sunset Yellow and Ponceau 4R (Sigma-Aldrich, Germany) with a concentration of 1 g L^{-1} were prepared by dissolving of 0.0500 g of reagents in 50 mL of deionized water. Solutions with a lower concentration of dyes were prepared by diluting the stock solutions with deionized water.

Polyhexamethylene guanidine chloride (PHMG) with the purity of over 95 % was purchased from Institute of Eco-Technological Problems (Moscow, Russian Federation). The PHMG solution was prepared by dissolving an accurately weighed portion in deionized water.

Aluminum oxide 150 basic purchased from Merck (particle size of 0.063–0.160 mm; pore diameter of 15 nm; specific surface area of 256 m^2 g^{-1}) was used as a matrix for the adsorbent synthesis.

Inorganic acids HCl, HNO_3 of analytical grade, which were additionally purified by distillation using a distillacid™ BSB-939-IR (Berghof, Germany) were used throughout the experiments. Solutions of acids with lower concentrations were prepared by diluting the concentrated acids with deionized water.

Modification of alumina surface with polyhexamethylene guanidine. In order to activate the surface, a portion of alumina was soaked in NaOH solution at pH 9 for 2 h, and then washed with deionized water until pH value reached 7. Then, 100 mL of PHMG solution (5 %) was added dropwise for 60 min to 10 g of aluminum oxide under continuous stirring. The adsorbent (Al_2O_3–PHMG) was washed with deionized water until the absence of positive reaction to PHMG in the rinsing water and dried at 40 °C. The presence of PHMG in the rinsing water was determined visually by the test with bromophenol blue.

Dye adsorption procedure. The adsorption of dyes in a batch mode was studied at the room temperature in glass graduated test tubes with a capacity of 15 mL. A solution containing 10 μg of a dye was placed into the test tube. Then a solution of HCl, NaOH or an acetate buffer solution was added to adjust the required pH value, distilled water was added to a total volume of 10 mL, and 0.100 g of Al_2O_3–PHMG adsorbent was introduced. The test tubes were capped and shaken for 1-30 min. The adsorbent was separated from the solution by decantation, transferred to a fluoroplastic cell, excess moisture was removed with filter paper, and the diffuse reflectance coefficient (*R*) was measured. The concentration of dyes in the aqueous solution before and after adsorption was determined by the spectrophotometric method using a characteristic absorption band (Table 1).

To construct adsorption isotherms at the room temperature, dye solutions with concentrations from 1.0 to 380.0 mg L^{-1} were placed into test tubes and pH was adjusted to 2.0, and water was added to the total volume of 10 mL. Then 0.100 g of Al_2O_3–PHMG adsorbent was added, the test tubes were capped and shaken for 10 min. After adsorption, the solutions were decanted and the residual content of dyes in them was determined.

Adsorption-spectroscopic determination of dyes in samples. The sample with pH 1.0 was placed in 15 mL test tubes with ground stoppers and 0.100 g of Al_2O_3–PHMG adsorbent was added. The test tubes were closed with stoppers and stirred for 10 min. The solution was decanted, the adsorbent was transferred to a fluoroplastic cell, excess water was removed with filter paper, and the diffuse reflectance coefficient was recorded using the characteristic wavelength for each dye (Table 1). The concentration of the dye was determined using a calibration curve.

RESULTS AND DISCUSSION

Characterization of Al_2O_3–PHMG adsorbent. The amount of fixed PHMG, determined from thermogravimetric analysis, was 8.9 mg (0.89 wt.%) per 1 g of

Table 1

The values of λ_{max} of food dyes under various conditions

Таблица 1

Значения λ_{max} пищевых синтетических красителей при различных условиях

Synthetic dyes	C_{HCl}, pH	λ_{max}, nm	
		Solution	Al_2O_3–PHMG
Sunset Yellow	0.1 M HCl	480	470
	6.5	480	470
	8.5	480	470
Ponceau 4R	0.1 M HCl	506	510
	6.5	506	510
	8.5	506	510

adsorbent. Taking into account the molecular weight of the monomeric fragment of PHMG (142 g mol^{-1}), the concentration of anion exchange centers (NH_2^+=) on the surface of the adsorbent was 63 µmol g^{-1}.

Pre-concentration of Sunset Yellow and Ponceau 4R. Al_2O_3–PHMG extracted Sunset Yellow and Ponceau 4R dyes from aqueous solutions in a wide range of pH of 1.0–8.0. Quantitative extraction (99 %) of dyes was observed in the pH ranges of 1.0–2.0 and 5.0–6.0 (Fig. 1 curves *1, 2*). Similar dependencies were obtained earlier during the extraction of the Fast Green FCF dye using the Al_2O_3-PHMG adsorbent [23].

The decrease in the extraction of dyes at pH 2-4 was probably due to a change in their form in solution (for the azo group –N=N− of dyes pK_a = 2.9±0.2), as well as a change in the mechanism of fixation of PHMG on the surface of aluminum oxide. With the increase in pH, the number of basic centers on the surface of the Al_2O_3 oxide also increased, and the number of Brønsted and Lewis acid centers decreased.

The time of attainment of adsorption equilibrium was10 min.

In the process of adsorption of dyes, the adsorbents are colored in bright orange (SY) and bright red (P4R), which is typical for the dyes. The diffuse reflectance spectra of Sunset Yellow and its maxima are shifted by 10 nm to the short-wave region relative to the absorption spectrum of its aqueous solution. The diffuse reflectance spectra of Ponceau 4R on the adsorbent surface are identical to the absorption spectra in solution. The maximum of the diffuse reflectance spectrum band is located at 470 nm and 510 nm for SY and P4R, respectively (Fig. 2). The spectral characteristics are constant over the entire pH range studied.

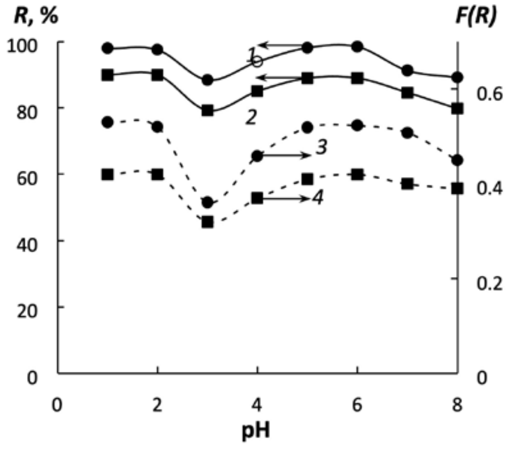


Fig.1. Dependences of recovery of Sunset Yellow (*1*) and Ponceau 4R (*2*) dyes by the Al_2O_3–PHMG and F(R) of Sunset Yellow (*3*) and Ponceau 4R (*4*) on the surface of the adsorbent vs. pH (0.1 g Al_2O_3-PHMG, C_{dyes} = 1 mg L^{-1}, *V*=10 mL, *t* = 10 min)

Рис. 1. Влияние pH на степень извлечения красителей Желтый солнечный закат (*1*) и Понсо 4R (2) сорбентом Al_2O_3-ПГМГ и F(R) Желтого солнечного заката (*3*) и Понсо 4R (*4*) на поверхности сорбента от pH (0.1 г Al_2O_3-ПГМГ, $C_{красит}$ = 1 мг/л, *V* = 10 мл, *t* = 10 мин)

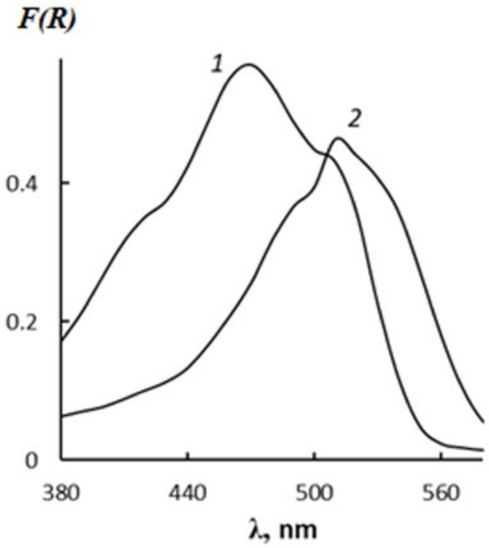


Fig. 2. Diffuse reflectance spectra of Sunset Yellow (*1*) and Ponceau 4R (2) dyes on the surface of Al_2O_3–PHMG adsorbent (0.1 g Al_2O_3-PHMG, C_{dyes} = 1 mg L^{-1}, *V*=10 mL, *t* = 10 min)

Рис. 2. Спектры диффузного отражения красителей Желтый солнечный закат (*1*) и Понсо 4R (*2*) на поверхности сорбента Al_2O_3-ПГМГ (0.1 г Al_2O_3-ПГМГ, $C_{красит}$ = 1 мг/л, *V* = 10 мл, *t* = 10 мин)

The maximum color intensity of SY and P4R dyes on the adsorbent surface is observed at pH 1.0–2.0 and pH 5.0–6.0 (Fig. 1 curves *3, 4*), which corresponds to their quantitative extraction. The contact time of the phases, necessary for the development of the maximum color of the adsorbents, is 10 min in all cases.

The adsorption capacity of Al_2O_3–PHMG for SY, calculated from the horizontal sections of adsorption isotherms, was 25 µmol g^{-1} at pH 6.0 and 95 µmol g^{-1} at pH 1.0 (Fig. 3). Such a difference in the adsorption capacity at different pH values is associated with a different arrangement of the dye on the surface of the adsorbent. Most likely, at pH 1.0, the SY, having only one deprotonated sulfo group, was fixed on the Al_2O_3–PHMG surface due to electrostatic interactions between the positively charged amino groups of the adsorbent surface and the negatively charged sulfo groups of the dye. In addition, the adsorption capacity at pH 1.0 for dye is higher than the number of anion exchange centers on the surface of the adsorbent. Under these conditions, the extraction of dyes could occur not only due to electrostatic interactions between the dye and the fixed PHMG, but also due to the interaction of the dye with free Lewis acid centers of the Al_2O_3 surface. At pH 6.0, the hydroxyl groups of the dye were partially deprotonated and the SY was also fixed due to the interaction of the hydroxo groups of the dye with the amino groups of polyhexamethylene guanidine.

The P4R adsorption isotherms on Al_2O_3–PHMG, constructed at pH 1.0 and pH 6.0, had a shape similar to that of SY dye (Fig. 3). The adsorption capacity determined from the horizontal section of adsorption

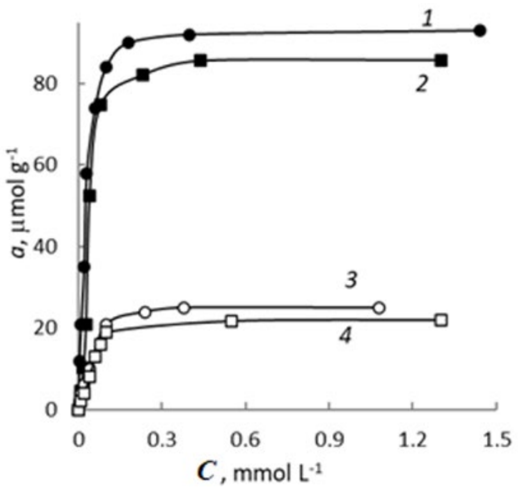


Fig. 3. Adsorption isotherms of Sunset Yellow (*1, 3*) and Ponceu 4R (*2, 4*) dyes on Al_2O_3-PHMG at pH 1.0 (*1, 2*) and pH 6.0 (*3, 4*)

Рис. 3. Изотермы адсорбции красителей Желтый солнечный закат (*1, 3*) и Понсо 4R (*2, 4*) на Al_2O_3-ПГМГ при pH 1,0 (*1, 2*) и pH 6,0 (*3, 4*)

isotherms was 22 μmol g^{-1} at pH 6.0 and 85 μmol g^{-1} at pH 1.0. These values are comparable with the adsorption capacities of the adsorbent in relation to the SY at the same pH values, which is associated with a similar structure of these dyes.

The distribution coefficient of dyes in the Henry region depend on the pH of the solution and was ~ $2 \cdot 10^2$ at pH 6 and ~ $1.6 \cdot 10^3$ at pH 1.

Adsorption-spectroscopic determination of dyes Sunset Yellow and Ponceau 4R dyes using Al_2O_3-PHMG. An increase in the concentration of dyes on the surface of the adsorbent leads to a proportional increase in the intensity of its color and values *F*(*R*) at λ = 470 nm for a SY and at λ = 510 nm for P4R.

Table 2

Analytical characteristics of adsorption-spectroscopic determination of synthetic dyes using Al_2O_3-PHMG (m_{ads} = 0.100 g, *V*=10 mL, pH 1, *n* = 5, *P* = 0.95)

Таблица 2

Аналитические характеристики сорбционно-спектроскопического определения синтетических красителей с использованием Al_2O_3-ПГМГ ($m_{сорб}$ = 0.100 г, *V*=10 мл, pH 1, *n* = 5, *P* = 0.95)

Synthetic dyes	The equation of calibration curve[a]	Linearity, mg L^{-1}	LOD[b], mg L^{-1}	s_r[c]
Sunset Yellow	$\Delta F(R) = 0.531C$ ($r = 0.994$)	0.02-10.0	0.005	0.07
Ponceau 4R	$\Delta F(R) = 0.423C$ ($r = 0.997$)	0.02-11.0	0.007	0.09

[a] - Δ*F*(*R*) is the difference between *F*(*R*) of the adsorbent with the dye and *F*(*R*) of the pure adsorbent sample, *C* is the dye concentration, mg L^{-1}.

[b] - the detection limit

[c] - when the content of dyes being determined is 10 times higher than the detection limit

The equations describing the calibration dependences, the range of the determined contents and the detection limit of the dyes at pH 1.0 are given in Table 2.

Dyes are usually used to add color to foods that contain a lot of sugar and citric acid, as well as various preservatives, so the interfering effect of these compounds has been studied. The selectivity of the determination of dyes using the example of a SY was studied at pH 1.0 and pH 6.0. Concentrations of additives that did not interfere with the determination of dyes are given in Table 3.

Selectivity of the determination of a SY at pH 1.0 was higher than at pH 6.0. This was due to the fact that the preservatives used were organic carboxylic acids, which at pH>5 were extracted by the Al_2O_3–PHMG simultaneously with the dyes. Thus, preservative molecules occupied the surface of the adsorbent and reduced the degree of dye extraction. Determination of 0.2 mg L^{-1} dyes at pH 1.0 did not interfere with 1000-fold excesses of glucose, ascorbic and citric acids, 100-fold excesses of benzoic and sorbic acids.

Determination of dyes in real samples. The proposed adsorption-spectroscopic method was applied for determination of SY and P4R dyes in beverages and jelly.

The highly carbonated beverages Mirinda (Manufacturer: OOO AK AKVALIF, Russia, Moscow Region, Noginsky District, Noginsk Industrial Zone - 23, Ground No. 1, Section 1, Building 2) and Iron Brue (Manufacturer: MEGAPAK LLC, Leninsky District, Vidnoye, B3 GIAP industrial zone, building 452) were chosen as real samples. The following substances are indicated in the composition of beverages: drinking water, sugar, acidity regulators (E330, E381), food flavoring, preservative (E211), caffeine (for “Iron Brue”), dyes. The beverages were degassed by ultrasonication. Then samples of beverages were filtered with 0.45 μm filter membrane and HCl was then added to pH 1.0. Ten milliliters of the resulting solution were taken for the analysis. The results of determination are shown

Table 3

Additive concentrations that do not interfere with the adsorption-spectroscopic determination of synthetic food dye Sunset Yellow using Al_2O_3-PHMG adsorbent

Таблица 3

Концентрации добавок, не мешающие адсорбционно-спектроскопическому определению синтетического пищевого красителя Желтого солнечного заката с использованием адсорбента Al_2O_3-ПГМГ

Interfering substances	Concetration, g L^{-1}	
	pH 1	pH 6
Ascorbic acid	50	15
Glucose	50	15
Benzoic acid	2.0	0.5
Citric acid	40	10
Sorbic acid	3.0	0.7

Table 4

Results of determination of Sunset Yellow in beverages using Al_2O_3-PHMG adsorbent (*n* = 3, *P* = 0.95)

Таблица 4

Результаты определения Желтого солнечного заката в напитках с использованием сорбента Al_2O_3-ПГМГ (*n* = 3, *P* = 0.95)

Sample	Added, mg L^{-1}	Found, mg L^{-1}
«Mirinda»	0	5.0 ± 0.2
	4.0	8.9 ± 0.5
«Iron Brue»	0	2.2 ± 0.1
	2.0	4.3 ± 0.2
	4.0	6.3 ± 0.3

in Table 4. The accuracy of the results was confirmed by the analysis of spiked samples of beverages.

The adsorption-photometric method was used for determination of dyes in jelly “Snegovichok” (Manufacturer: LLC Siberian Dairy Products Plant, Russia, Novosibirsk region, Berdsk, Eastern Industrial Zone) with peach flavor (SY) and strawberry flavor (P4R). The products include: drinking water, sugar, food additives: stabilizer (gelatin, pectin), acidity regulators (citric acid, ascorbic acid), potassium sorbate preservative, flavors, dyes. Before the analysis, 4.0 g of jelly was weighed in a 100 mL beaker, then 50.0 mL of distilled water was added, stirred and heated in a water bath to a temperature of 35 °C. Hydrochloric acid was then added to pH 1.0. 10.0 mL of the resulting solution was taken for the analysis. The results of determination are shown in Table 5. The accuracy of the obtained results was confirmed by the analysis of spiked solutions.

Table 5

Results of determination of Sunset Yellow and Ponceau 4R in jelly using Al_2O_3-PHMG adsorbent *(n* = 5, *P* = 0.95)

Таблица 5

Результаты определения красителей Желтого солнечного заката и Понсо 4R в желе с использованием сорбента Al_2O_3-ПГМГ (*n* = 5, *P* = 0.95)

Sample (dye)	Added, μg	Found, μg
Jelly with peach flavor (SY)	0	5.6 ± 0.5
	5	10.3 ± 0.8
Jelly with strawberry flavor (P4R)	0	8.6 ± 0.7
	5	13.0 ± 0.9

CONCLUSIONS

A simple and rapid method for the determination of synthetic dyes using an adsorbent based on Al_2O_3 modified with polyhexamethylene guanidine was proposed. Al_2O_3-PHMG adsorbent extracted synthetic dyes Sunset Yellow and Ponceau 4R in a wide range of acidity, pH = 1.0–8.0. Quantitative extraction of dyes was observed in the pH ranges of 1.0–2.0 and pH 5.0–6.0. The adsorption capacity of the proposed adsorbent at pH 1 was 95 and 85 μmol g^{-1} for Sunset Yellow and Ponceau 4R, respectively. The methods of adsorption-spectroscopic determination of synthetic dyes with a LOD of 0.005 mg L^{-1} (Sunset Yellow) and 0.007 mg L^{-1} (Ponceau 4R) have been developed. The proposed method was used in the analysis of synthetic food dyes in beverages and jelly.

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