SIMULTANEOUS DETERMINATION OF SYNTHETIC DYES IN GUMMY CANDY USING NOVEL MESOPOROUS MAGNETIC GRAPHENE OXIDE@ZEIN AEROGEL FOLLOWED BY A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTOR

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ABSTRACT

The present work aimed to determine colorants used as food additives to confirm the gummy candy products authentication using a newly designed adsorbent in magnetic dispersive solid phase extraction. Nowadays, many kinds of synthetic dyes are widely added to food products and should be strictly monitored and regulated like other food additives. A mesoporous aerogel magnetic graphene oxide@zein adsorbent was prepared in a template-free and facile self-assembling manner for magnetic dispersive solid phase extraction extraction of 8 frequently used synthetic dyes in gummy candy samples. The adsorbent was designed and successfully characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, vibrating-sample magnetometry, and Brunauer–Emmett–Teller analysis. The isotherm parameters were calculated, and the amount of colorant was determined using high performance chromatography. The proposed method was validated according to the FDA guidelines over the concentration range of 0.05-50 µg/mL. The maximum adsorption capacities of the adsorbent for Tartrazine, Quinoline Yellow, Ponceau 4R, Sunset Yellow, Allura Red, Carmoisine, Indigo Carmine, and Brilliant Blue FCF were 43.3, 43.2, 26.9, 37.8, 38.3, 74.5, 28.3, and 19.9 mg g^{-1}, respectively. Limits of detection (LODs) were from 5 to 20 ng/mL. Inter- and intra-day precisions and accuracies were within 10%. The developed method was proved specific to determine synthetic colorants in gummy candy samples and can be easily used in food quality control laboratories.

Keywords: Aerogel, Magnetic micro dispersive solid phase extraction, Zein, Food additive, Mesoporous

INTRODUCTION

Food additives are chemical substances added to food to improve its taste, texture, and flavor. Color additive is any certified substance added to food products to imparte in the food palatability. Food dyes fall into two large categories; natural dyes and synthetic dyes. However, most dyes with natural origin are unstable and can quickly lose their quality during food processing. Nowadays, many synthetic dyes are widely added to food products due to their low price, excellent efficiency, cost-effectiveness, chemical stability, and low microbiological contamination compared to natural dyes **(Alves, Brum et al. 2008)**. Some concerns indicate that dyes and their metabolites pose adverse health effects to humans, including allergic symptoms, DNA damage, hyperactivity and carcinogenesis **(Zou, He et al. 2013, Rovina, Prabakaran et al. 2016)**. Azoreductase enzymes produced by intestinal bacteria can reduce Azocolorants and in liver cells releasing aromatic amines into the body organism **(Rafii, Hall et al. 1997)**. This may cause headaches in adults while children often bother from distractedness and hyperactivity **(Hawley and Buckley 1976)**. Therefore, the usage of these synthetic dyes is restricted worldwide and leads to obtaining knowledge about food components **(Sun, Sun et al. 2013)**.

In Iran, the use and maximum limit of colorants in foods are controlled by the Food and Drug Administration, based on a legislative resolution. The following water-soluble synthetic colorants are permitted in food in Iran: Sunset Yellow (INS110), Carmoisine (INS122), Ponceau 4R (INS124), Quinoline Yellow (INS 104), and Allura Red (INS129). The permitted food colorants that are not azo are Indigo Carmine (INS132) and Brilliant Blue FCF (INS133). Tartrazine (INS102) is an azo dye that is not permitted in foodstuff in Iran.

There is a high demand that each substance should be labeled correctly and be at its accepted level of use. However, all food additives and food ingredients should be claimed on the foodstuff label. Consumers should feel comfortable and safe about the food they eat. Therefore, the determination of synthetic dyes in food products will be highly valuable to monitor the quality and safety of dye substances added to food products. In complex food matrixes, an accurate and straightforward sample preparation method is needed.

Currently, high-performance liquid chromatography (HPLC) methods coupled with ultraviolet/visible detector (UV/vis), diode-array detectors (DAD) **(Minioti, Sakellariou et al. 2007, Al-Degs 2009, Enríquez-Gabeiras, Gallego et al. 2012, Olgun, Ozturk et al. 2012, Bonan, Fedrizzi et al. 2013)** and mass spectrometry (MS) **(Holčapek, Volná et al. 2007, Feng, Zhao et al. 2011, Zou, He et al. 2013)** are reported to analyze synthetic colorants. In addition, many sample preparation methods, such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are used to isolate synthetic dyes from different foodstuff **(Ma, Luo et al. 2006, Feng, Zhao et al. 2011)**. The utilization of extraction devices such as cartridges, which are expensive and recommended for single use, is a significant disadvantage associated with the SPE method. LLE is also practiced with a large volume of hazardous solvents. Furthermore, these methodologies are tedious and time-consuming. Fortunately, several microextraction methods based on miniaturization of chemicals and materials consumed have emerged to obtain more effective extraction processes.

Compared to the established SPE techniques, dispersive micro solid-phase extraction (DMSPE) has recently attracted considerable attention as a new sample preparation technique. In this methodology, as-prepared adsorbents are dispersed into the sample solution, and then target analytes can be loaded on it and eluted by an appropriate solvent in microliter size.

In recent years, nanomaterials have attracted considerable attention as adsorbent, owing to their unique physical and chemical properties. Several nanomaterials, such as graphene oxide, superparamagnetic surface molecularly imprinted nanoparticles **(Madrakian, Afkhami et al. 2013)**, zein nanoparticles **(Farhadi, Matin et al. 2014)**, and carbon nanotubes **(Stafiej and Pyrzynska 2008)**, and their functionalized products have been reported as solid-phase adsorbents in the DMSPE process. These structures have a small size and show an increased surface-to-volume ratio giving them different thermal, mechanical, and electronic properties. Applying magnetic or magnetizable materials compared to other materials in the magnetic solid phase extraction process does not need complicated extraction and separation processes **(Chen, He et al. 2018)**. Adsorbents with magnetic materials were separated from the solution by an external magnetic field, preventing column clogging caused by particle residues.

In addition, the type of materials associated with the magnetic core can be specifically selected, enhancing the selectivity and extraction efficiency considerably. At present, the nanocomposite of Fe_3O_4 and reduced graphene oxide was used to extract four colorants from beverage by magnetic solid-phase dispersion extraction **(Wang, Chen et al. 2015)**.

Zein, is an inexpensive natural by-product of the bioethanol industry, known for its biodegradable and biocompatible properties. Zein proteins are hydrophobic and water-insoluble **(Patel and Velikov 2014)**. Magnetic nanomaterials have gained attention due to their role in the separation field. Aerogel is a porous material with a porosity of 80.0% to 99.8% which its structure can be controlled and tuned. Carbon aerogels, as three-dimensional materials, have been known owing to their pivotal properties such as high surface area and porosity compared to two-dimensional materials **(Chisvert, Cárdenas et al. 2019)**. Porous materials are subdivided into micro (pore diameters less than 2 nm), meso (pore diameters between 2 nm and 50 nm), and macro size (pore diameters greater than 50 nm) according to the International Union of Pure and Applied Chemistry (IUPAC). Porous materials with different channels and tunable surface holes can be produced by the self-assembling method **(Ren, Hui et al. 2013, Han, Wang et al. 2014)**. Carbon aerogel has a very low density, and it is not easy to separate its particles from the sample solution; therefore, a magnetic carbon aerogel is highly promising. The combination of zein and magnetic carbon-based materials provides an efficient adsorbent as a composite material for food sample clean-up in the magnetic-dispersive micro solid phase extraction process (M-DMSPE). The present work aimed to determine colorants used as food additives i.e. INS numbers 102, 104, 110, 122, 124, 129, 132, and 133, to confirm the gummy candy products authentication. Magnetic GO@zein was prepared by the self-assemble method as a novel M-DMSPE adsorbent, and used for simultaneous extraction of eighth water-soluble dyes from gummy candy samples. The HPLC-DAD instrument was applied to quantify the levels of these dyes in gummy candy products in a single run. The developed method was validated according to the FDA guidelines for analysis in terms of precision, accuracy and recovery.

MATERIAL AND METHODS

Chemicals & solutions

Tartrazine (INS 102), Quinoline Yellow (INS 104), Ponceau 4R (INS 124), Sunset Yellow (INS 110), Allura Red (INS 129), Carmoisine (INS 122), Indigo Carmine (INS 132), and Brilliant Blue FCF (INS 133) purchased from Istaza Co. (Tehran, Iran). Methanol, ethanol, acetonitrile, ammonium acetate, FeCl3•6H2O, FeCl2•4H2O, hydrochloric acid (HCl), graphite powder, and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). All reagents and solvents were of analytical grade. Zein powder was purchased from Sigma (USA). Deionized water (Shahid Ghazi Company, Tabriz, Iran) was used for sample preparations. A Millipore membrane filter, 0.2 μm (Chromafil, Germany) was used to filtering all the chemicals and samples.

Standard solutions and samples

A stock standard solution of all dyes (1000 mg/L) was prepared in methanol and stored at 4 °C. Standard solutions for calibration purposes were prepared by freshly diluting appropriate amounts of the stock solution with deionized water. Ten samples of gummy candy with different flavors were purchased from local markets in Tabriz (Tabriz, Iran). All samples were stored in a refrigerator, at 4° C, until analysis.

Instrumentation & hplc condition

A Fourier transform infrared (FT-IR) spectrometer (Tensor 27, Bruker, Germany) was applied in the range of 400–4000 cm−1 to characterize the synthesized magnetic GO@zein. Scanning electron microscopy (SEM) MIRA3 FEG–SEM (Tescan, The Czech Republic) was utilized for the morphologic survey. Magnetization curves were recorded using a (VSM—4 in., Daghigh Meghnatis Kashan Co., Iran) at room temperature. Zeta potential measurements were performed using a Zetasizer (Nanotrac Wave, Microtrac, Germany). The Brunauer-EmmettTellet (BET) surface area was performed by Belsorp mini system.

An HPLC instrument (Agilent Technologies (Waldbronn, Germany)) equipped with a DAD was used for the analysis. Agilent Chemstation® (Waldbronn, Germany) software was used for data handling. Separation was performed on the C18 column (15 cm×4.6 mm×5 μm). The mobile phase composition was a mixture of (A) 0.1 M ammonium acetate (pH adjusted to 7.0 using hydrochloric acid) and (B) methanol: acetonitrile (80: 20) in a gradient program and applied at a flow rate of 1.2 mL/min. The gradient elution program is provided in Table 1. A spectrometer UV/VIS (Thermo, USA) was used for the acquisition of the target dyes absorption spectrum. The target dye's absorbance were set at 420–620 nm.

Table 1 Gradient program for separation of the synthetic food dyes

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
2	100	0
37	20	80
40	20	80
45	100	0
50	100	0

Mobile phase A is ammonium acetate 1% (pH 7.0) and mobile phase B is methanol:acetonitrile (80:20).

Synthesis of magnetic GO@ZEIN

In this work, we try to prepare GO-based porous material with strong mechanical properties by using zein, as the cross-linking agent. Zein has an amphiphilic characteristic and with incorporation with GO provides a low hydrophilicity structure. To prepare magnetic GO dispersion our previous work was applied. Resultant aqueous magnetic GO solution was sonicated for 30 min before use. Zein solution was separately prepared by dissolving 11 mg zein in the mixture of ethanol (2 mL) and water (1 mL) while sonicating for 5 min. Twenty mL of magnetic GO solution (5.0 μg/mL) was mixed with zein solution (3 mL) and sonicated for 5 min. Then, the resultant mixture was put into a Teflon reaction kettle and heated to 200 °C in an oven for 4 h. After that, the reaction container was cooled at room temperature, and obtained magnetic GO@zein adsorbent was rinsed with water and put under freeze-drying process.

Magnetic dispersive micro solid-phase extraction (M-DMSPE)

Gummy candy samples were finely smashed and 1 g of each sample was dissolved in 10 mL water for 30 min at 55 °C. Then, the sample was centrifuged and the supernatant was transferred into another test tube to perform the extraction step. The accurately weighed 8 mg of magnetic GO@zein was added into the sample solution, and put in an ultrasonic bath for 4 min. Then, the adsorbent was separated using an external magnetic field. Next, 500 μL of NaOH (0.5 M) was added to the adsorbent as desorption solvent, while the mixture was put again in an ultrasonic apparatus for 2 min. Finally, the upper phase was separated by discarding the solid phase by a magnet and loaded into HPLC-DAD for further analysis.

RESULTS AND DISCUSSION

Characterization of synthetic materials

FT-IR pattern of zein and magnetic GO@zein was observed in Fig. 1a. The characteristic absorptions of –OH stretching and –NH stretching at 2800–3500 cm^{-1}, –C = O stretching at ~ 1645 cm^{-1}, band at 1239 cm^{-1} assigned to the axial deformation vibrations of the C–N bond **(Li, Yin et al. 2013)**. A new peak at 1239 cm^{-1} emerged as C–N stretching vibrations of zein in the 3D magnetic GO@zein; therefore, we can deduce that zein was successfully assembled into the 3D network structure of adsorbent. Also, a revealed absorption peak at 1533 cm^{-1} corresponds to the N–H bending vibration of zein in the 3D magnetic GO@zein. A peak at 609 cm^{-1} is a confirmation of the successful synthesis of magnetic GO@zein.

Magnetization S-like graph in Fig. 1b, is proof of the superparamagnetization of the magnetic GO@zein with the Ms = 42.12 emu/g. This confirms that adsorbent is readily collected by an external magnetic field.

The SEM image of magnetic GO was observed in Fig. 1c. This Figure gives the information about the shape of the magnetic GO adsorbent. . The surface of lamellar magnetic GO is very smooth without any crack. Fe_3O_4 nanparticles are well distributed on the GO surface due to their intention to accumulate as agglomerates because of their high surface energy and dipole-dipole interaction between the particles. The SEM graph of the adsorbent after forming the porous structure is shown in Fig. 1d. As it is obvious, under high heat and pressure, zein incorporated through the magnetic GO layers and a cross-linked structure has been formed.
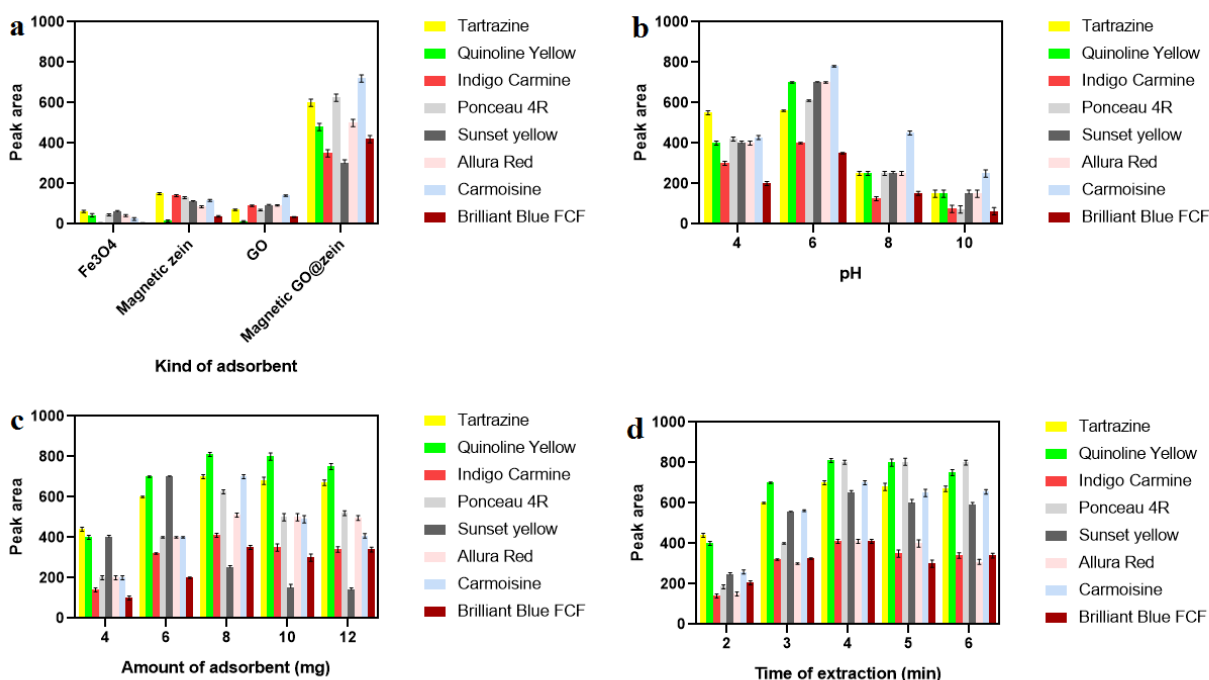
Surface area of porous adsorbent was estimated using BET Method. This method is an approach that computes the monolayer coverage of the adsorbent by the adsorption isotherm of nonreactive gas such as nitrogen under the defied range of pressure. Obtained results were transformed in a linearized BET graph, and the surface area was estimated. BET standard pressure region conventionally was calculated from 0.05 to 0.3 in the relative pressure (p/p0) axis with the theory that the monolayer formation is present in this range **(Sing 2013)**. After incorporating the zein in magnetic GO, the obtained surface area and porous size increased from 12 m^2/g to 225 m^2/g. This indicates that zein was successfully penetrated between the GO sheets and produced an interconnected network.
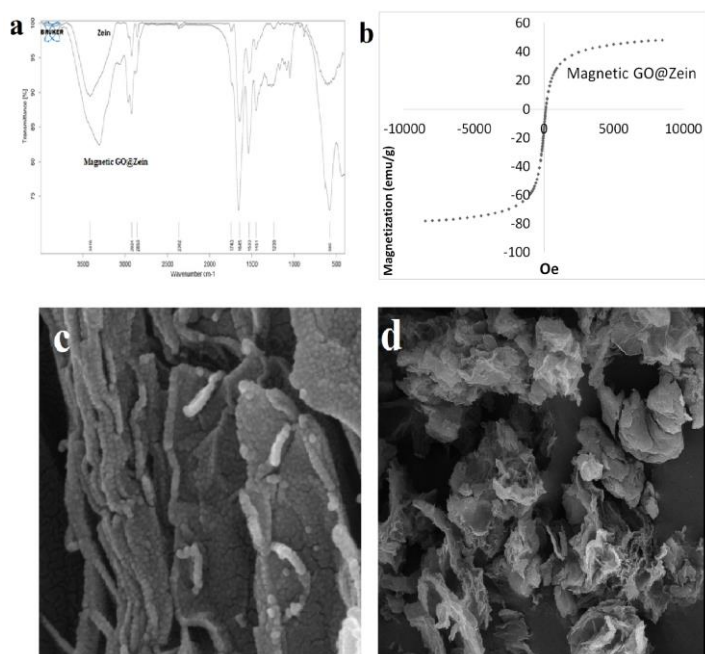
Figure 1 (a) Fourier transform infrared spectroscopy spectrums of magnetic GO@zein, (b) Vibrating sample magnetometry of magnetic GO@zein, (c) SEM images of magnetic GO, and (d) magnetic GO@zein

Optimization procedure

M-DMSPE provides a large and extended surface to extract the studied dyes. To have the best experimental conditions, the most important parameters influencing the extraction process should be checked. We used one parameter in a time method **(Barzegar and Hamidi 2017, Barzegar, Mousavi et al. 2017)** in which the parameters changed one by one, while the other parameters maintained constant. Analytical response (peak area) was recorded after each change, and experiments were performed in triplicates. Several parameters such as kind of adsorbent, sample pH, amount of adsorbent, adsorption time, type and volume of the desorption solvent, and desorption time were investigated.

To compare the ability of different adsorbents, the adsorption process was tried with other adsorbents, i.e., Fe_3O_4, GO, magnetic zein, and magnetic GO@zein. As Fig. 2a shows, the ability of the magnetic GO@zein in the extraction process is notable. As anticipated, due to the porous structure of adsorbent and interconnected architecture, there was an extended space to trap the dyes, and the extraction efficiency increased effectively. Magnetic GO@zein was synthesized under simple self-assembly conditions in binary solvents provided by the hydrothermal route. Our proposed method has some advantages: (1) Using an environmentally friendly and available zein biopolymer, we synthesized a 3D GO-based porous material. Zein has an amphiphilic characteristic helping to improve the mechanical power of the adsorbent; (2) In practice, compared to GO or magnetic zein, prepared adsorbent to disperse very well in sample media and shows low hydrophilicity; (3) Using our strategy, adsorbent morphology was well conserved after freeze-drying without collapse and scattering, which making a significant contribution to its powerful adsorption capacity.

Controlling the pH of sample media has a significant effect on the extraction performance. It determines the ionic status of adsorbents and adsorbates and other specious that may present in the sample. According to Zeta potential analysis, magnetic GO@zein carries a negative charge, and it is in the anionic form in the pH range of 4-8. Investigations illucude that more replusion forces cused to the stabilty of nanoparticles **(Chen, Fu et al. 2013)**. The effect of pH parameter on the extraction set-up was studied in the range of 3-8. Extraction capability was elevated by increasing the pH from 4 to 6 (Fig. 2b). Based on previous investigations, zein is almost soluble at alkaline pH **(Patel and Velikov 2014)**; therefore, elevating the pH up to 8, the assessable cites for extraction decreased, and suspension of adsorbent was more stable at pH 6. Regarding the presence of several aromatic rings in dyes structure, the potential of forming pi-pi interactions between adsorbent and dyes is high. Conjugated rings in adsorbent have electron distribution because of pi-pi bonds and threfore interact well with dyes.
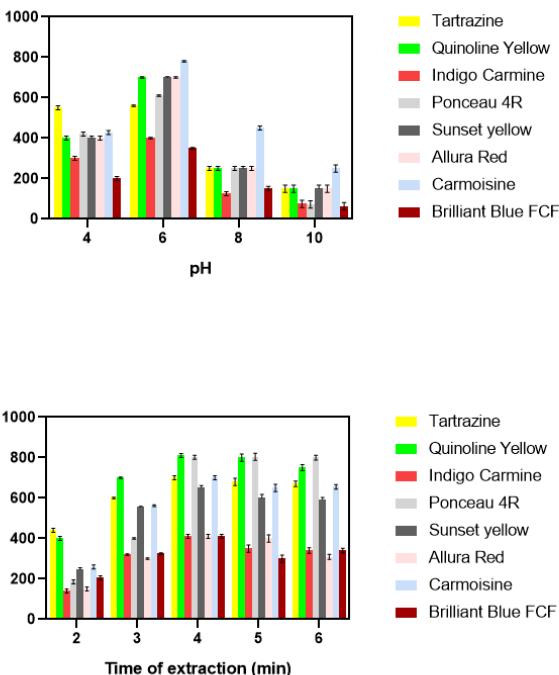

Figure 2 Optimization of a) kind of adsorbent, b) pH, c) amount of adsorbent and time of extraction, and (d) on the extraction of 8 synthetic dyes from gummy candy sample

The adsorption quantity of target dyes depends on the adsorbent's dosage since the adsorbent quantity provides the necessary sites for loading the dyes. In this regard, more available sites are beneficial for maximum extraction. To assess the influence of adsorbent amount on the extraction process, different amounts of adsorbent (4-12 mg) were tested (Fig. 2c). The obtained data revealed that by increasing the adsorbent amount up to 8 mg, the analytical response increased and further increase in adsorbent dosage did not show positive effects. Therefore, 9 mg of adsorbent is enough for the complete loading of target dyes. Compared to conventional SPE versions, M-DMSPE provides a wide surface for the adsorption process so that the loading process occurs with only 9 mg of adsorbent that is very economical. In a higher amount of the adsorbent, the extraction rate was decreased, which might be due to aggregation of the adsorbent.

Adsorption time shows an important effect on the extraction process since it determines the number of analytes loaded onto the adsorbent. In M-DMSPE, the extraction time was indicated as the time interval between the contact of the sample solution with the adsorbent and the beginning of the adsorbent separation. The extraction time was different from 2 to 6 min, and according to the analysis data (Fig. 2d), by giving 4 min to the extraction process, the analytical response was maximum.

One of the significant properties of M-DMSPE is that the equilibrium between analytes and the adsorbent occurs very fast. In a couple of minutes, the extraction process completed.

In M-DMSPE, desorption performance can be completed by changing the solution pH or by applying an appropriate organic solvent. Typical organic solvents such as acetonitrile, acetone, ethanol, 2-propanol, and methanol can be used as desorption solvents in the present work. As most of the dyes have acidic nature, the alkaline media application is beneficial in eliminating dyes from the adsorbent. Alkaline solution (NaOH 0.5 M) elutes the dyes better than other organic solvents (Fig. 3a). The volume of alkaline elution solvent was also checked. According to observations (Fig. 3b), 500 μL of NaOH presented a maximum extraction output. The ultra-sonication time was changed in the range of 2–5 min (Fig. 3c). The results indicated that 3 min was enough for equilibrium achievement and elution of target analytes from the adsorbent.
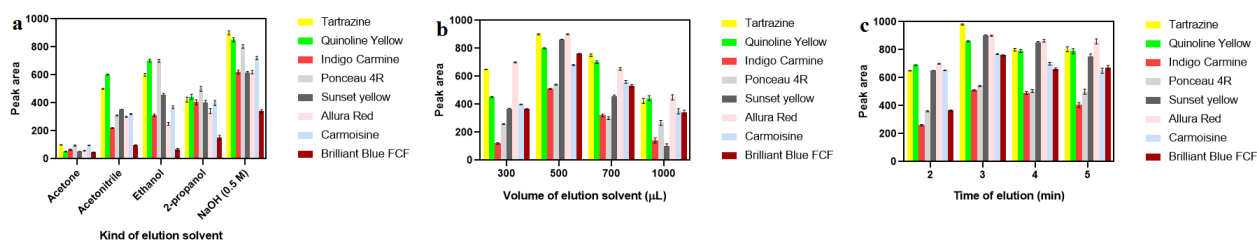
Figure 3 Optimization of (a) kind of elution solvent, (b) volume of elution solvent, and (c) time of desorption on the extraction of 8 synthetic dyes from gummy candy sample

Validation parameters

Analytical method validation is the guidance to confirm the procedure employed for target use. The *FDA* has issued a process for method validation **(FDA 2001)**. The present paper covers the aspects required for an analytical method, including linearity, accuracy, precision, and specificity.

Precision, accuracy, and recovery

The maximum absorption values were recorded for each dye and used for the chromatographic analysis with a DAD. The calibration curves were constructed separately for each dye separately in the range of 0.05 to 50 μg/mL. Table 1 shows the details of the mathematical equation in a linear range. Limit of detection (LOD) was defined as a signal-to-noise ratio (S/N) of three. Most signals were well resolved as the resolution values were more than 1.5.

Table 2 Linear equations, coefficients of determination (R^2), limits of detection (LOD), limits of quantification (LOQ), retention time (tR), λ Max and repeatability (intra-assay) RSD (%) of each synthetic colorant studied

Colorant	Linear range (μg/mL)	R^2	LOD (ng/mL)	tR (min)	λ Max (nm)	Repeatability (intra-assay) RSD (%)
Tartrazine	0.05-50	0.99	8	9.44	420	1.2
Quinoline Yellow	0.05-50	0.99	8	14.69	420	2.5
Ponceau 4R	0.1-50	0.99	10	14.24	515	7.2
Sunset Yellow	0.02-50	0.99	7	15.48	495	4.2
Allura Red	0.02-50	0.99	5	18.12	515	0.7
Carmoisine	0.02-50	0.99	5	22.51	515	1.7
Indigo Carmine	0.07-50	0.99	20	11.65	620	5.5
Brilliant Blue FCF	0.01-50	0.99	5	24.51	620	6.7

The method accuracy and precision were evaluated using three samples in three different concentrations in one dye and five different days to assess the whiten and between day experiments, respectively. The details of precision and accuracy experiments in Table 3 confirm the method's acceptance according to the FDA guidelines.

Table 3 Accuracy and precision of the method

Colorant	Concentration (μg/mL)	Assay precision (RSD %) Intra-day	Assay precision (RSD %) Inter-day	Assay accuracy (%)
Tartrazine	10	1.2	4.9	2.0
	30	2.5	5.3	1.2
	50	0.7	4.0	2.5
Quinoline Yellow	10	1.5	6.0	5.2
	30	5.0	4.6	4.7
	50	0.2	2.5	0.5
Ponceau 4R	10	3.5	5.9	6.2
	30	2.0	2.9	3.2
	50	0.1	6.0	1.0
Sunset Yellow	10	0.6	4.5	2.0
	30	0.8	2.3	5.3
	50	0.6	2.0	2.0
Allura Red	10	5.3	5.3	3.2
	30	2.4	2.9	4.2
	50	1.9	3.0	0.7
Carmoisine	10	4.2	6.2	5.5
	30	2.0	4.3	2.4
	50	0.5	5.2	0.9
Indigo Carmine	10	4.8	4.5	3.8
	30	5.3	1.8	2.5
	50	1.2	2.2	1.3
Brilliant Blue FCF	10	4.9	4.2	4.0
	30	5.2	1.5	4.0
	50	1.3	1.9	0.6

The method accuracy was also calculated as the mean percent recovery and estimated using three concentrations of the samples (Table 4).

Table 4 Average percentage of recovery (%) and SD values for each concentration level of artificial colorant analyzed (n=3)

Colorant	10 (µg/mL)	30 (µg/mL)	50 (µg/mL)
Tartrazine	98.1±1.3	105.1±2.5	95.0±1.2
Quinoline Yellow	102.5±1.7	112.6±1.7	108.3±0.4
Ponceau 4R	102.0±0.7	100.3±1.3	97.0±0.8
Sunset Yellow	105.2±1.2	105.6±0.7	114.0±0.9
Allura Red	97.5±2.7	104.3±1.2	107.2±1.7
Carmoisine	93.0±2.0	99.3±1.5	95.0±2.5
Indigo Carmine	100.2±1.2	95.2±0.5	98.5±0.3
Brilliant Blue FCF	98.0±1.3	112.2±0.9	110.3±0.1

The data presented that the recovery values for the analytes were in the range of 93.0–114.0%, and these values were within the acceptable range, confirming that the developed method could simultaneously examine 8 food colorants with better recovery.

The retention time (tR) of each colorant showed that separation was completed within 28 min. Therefore, the analysis time was extended for few minutes, providing the stabilization time of the HPLC column and preparing the set-up for the following analysis.

Isotherm studies

If adsorbent and adsorbates interact with each other long enough, an equilibrium is defined between the amount of adsorbate loaded on the adsorbent and the adsorbent remained in the sample solution. The equilibrium formulas are established by isotherms. In this work, two well-known models of Langmuir and Freundlich equilibrium isotherms were used to describe the experimental adsorption data in an equilibrium state (**Wang, Wang et al. 2013, Altintig, Onaran et al. 2018**). The Langmuir isotherm model was used to estimate the maximum adsorption capacity q_m (mg g^{-1}) corresponding to complete monolayer coverage on the adsorbent surface. The Langmuir KL value was given to the adsorbate affinity to the adsorption sites.

$1/n$ is the heterogeneity factor of the Freundlich model, and its smaller value than 1 (n>1) demonstrates the intensity of adsorption (**Jin, Yu et al. 2014**). K_F is directly proportional to the adsorption capacity.

The dyes uptake capacity (q_e) of the adsorbent was evaluated using the following equation:

$$q_e = \left(\frac{(C_0 - C_e)V}{m}\right) \times 100$$

Where C_0 and C_e are the initial and equilibrium concentrations of each dye solution (µg/mL), respectively, V is the volume of the sample (mL), and m is the mass of the magnetic GO@zein adsorbent (g).

Table 5 shows the detail of isotherms according to the higher correlation value of the Langmuir model fitting the experimental data.

Table 5 Constant parameters, correlation coefficient and error analysis for different adsorption isotherm models for the adsorption of dyes on magnetic GO@zein. (initial dyes concentration = 10–50 µg/mL, adsorbent mass = 0.008 g, pH = 6.0, volume = 10 mL, sonication time = 4 min and T = 25° C)

	Langmuir isotherm model $\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m K_L}$ Plot: Ce/qe versus Ce			Freundlich isotherm model $\ln q_e = \ln K_F + \frac{1}{n_F} \ln C_e$ Plot: ln qe versus ln Ce		
Dye	q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	$1/n_F$	K_F (mg g^{-1})	R^2
Tartrazine	43.35	0.20	0.99	0.46	9.39	0.98
Quinoline Yellow	43.20	0.17	0.96	0.53	7.68	0.85
Indigo Carmine	28.33	0.08	0.97	0.56	3.31	0.94
Ponceau 4R	26.94	0.15	0.99	0.44	5.42	0.91
Sunset Yellow	37.80	0.06	0.98	0.63	2.95	0.97
Allura Red	38.31	0.29	0.97	0.39	10.75	0.96
Carmoisine	74.59	0.73	0.99	0.60	28.81	0.96
Brilliant Blue FCF	19.99	0.19	0.93	0.38	4.97	0.87

Real samples

The proposed method was applied to assay the concentration levels of Tartrazine, Quinoline Yellow, Ponceau 4R, Sunset Yellow, Allura Red, Carmoisine, Indigo Carmine, and Brilliant Blue FCF in gummy candy samples collected from the Iranian market.

Table 6 shows, 4 out of the 8 colorants were detected at least once. The concentrations of colorants in gummy candy samples ranged from 3.04 (Sample 10) to 102.64 µg/mL (Sample 9). Sample 9 had the highest amount of colorant among the others, containing 102.64 µg/mL of Tartrazine which is banned in Iran. Interestingly, some manufacturers did not report that their products included a particular colorant; however, our method determined a different fact. For example, in samples 9 and 10 the colorant Tartrazine was identified, whereas it was not declared on the product label.

Among the samples assayed, there was one (sample 8), which the manufacturer declared that no artificial colorant had been used. The proposed method confirmed the absence of a synthetic colorant, and the values were below the detection limit of the proposed method. In other cases, the most commonly used dye was Allura Red, having been identified in 70% of samples in the range from 3.79 to 24.45 µg/mL.

Finally, the proposed method was compared to other published methods in terms of sample treatment strategies. Table 7 compares the information of the proposed approach to the reported methods concerning the linear range and LOD. Reported LLE and SPE methods to extract some food colorants from foodstuff is tedious and time-consuming (**Fuh and Chia 2002, Huang, Shih et al. 2002**). These methods suffer from consuming a large number of hazardous solvents, which are neither user-friendly nor environmental-friendly. In some more modern methods such as ionic liquid dispersive liquid-phase microextraction proposed to extract some dyes, some disadvantages have been observed (**Wu, Guo et al. 2013**). The separation of the pure organic phase remains an essential obstacle in liquid phase extraction methods. The magnetic dispersive solid phase extraction method was reported to extract four dyes while our process could extract eight dyes simultaneously (**Wang, Chen et al. 2015**). In another study, zein-modified magnetic Fe$_3$O$_4$ nanoparticles were prepared and used for the micro-solid-phase extraction of azo dyes, including carmoisine, ponceau 4R, sunset Yellow, and Allura red from food dyes (**Jangju, Farhadi et al. 2017**).

Table 6 Artificial colorants identified and quantified in real samples of gummy candy

Sample #	Label	Colorant found	Concentration µg/mL
1	Brilliant Blue FCF	Brilliant Blue FCF	3.73
2	Brilliant Blue FCF	Brilliant Blue FCF	4.11
	Allura Red	Allura Red	24.45
3	Brilliant Blue FCF	Brilliant Blue FCF	9.65
	Allura Red	Allura Red	13.34
4	Allura Red	Allura Red	8.22
5	Allura Red	Allura Red	20.46
	Tartrazine	Tartrazine	12.83
6	Allura Red	Allura Red	9.42
7	Allura Red	Allura Red	12.14
8	Natural	-	-
9	-	Brilliant Blue FCF	26.30
		Tartrazine	102.64
		Sunset Yellow	10.49
10	-	Allura Red	3.79
		Tartrazine	3.04

Our proposed adsorbent shows superiority over other adsorbents due to the application of 3D material as an extraction adsorbent for the simultaneous extraction of 8 dyes, leading to maximum interaction between the analyte adsorbent, thereby maximizing the extraction efficiency. A facile hydrothermal method was used to synthesize the 3D magnetic GO@zein adsorbent. All these proofs confirm that the proposed M-DMSPE and HPLC–DAD are an applicable and simple technique that can successfully be applied for the pre-concentration and determination of 8 synthetic dyes in gummy candy samples. The results proved that the properties of the adsorbent were excellent to extract synthetic dyes with low cost and much less contamination. The method developed in this study was applicable, especially in developing countries.

Table 7 Comparison of the proposed method with other methods reported in the literature

Method	Food dye	LOD[a] (ng/mL)	Linear dynamic range (μg/mL)	Ref.
ILDLPM/HPLC[b]	Sunset Yellow, Allura red, Ponceau 4R	0.32	-	**(Wu, Guo et al. 2013)**
MSPDE/HPLC[c]	Tartrazine, Allure red, Amaranth, New coccine	11.9	0.05–10	**(Wang, Chen et al. 2015)**
LLE[d]/HPLC (ion-pair)	Sunset Yellow, Allura red, Ponceau 4R	10	0.05–10	**(Fuh and Chia 2002)**
PA-SPE/HPLC[e]	Sunset yellow, Allura red	500	-	**(Huang, Shih et al. 2002)**
DM-μ-SPE/HPLC	Sunset Yellow, Allura red, Ponceau 4R, Carmoisine	0.3–0.9	0.003–1.0	**(Jangju, Farhadi et al. 2017)**
M-DMSPE	Tartrazine, Quinoline Yellow, Ponceau 4R, Sunset Yellow, Allura Red, Carmoisine, Indigo Carmine and Brilliant Blue FCF	5	0.05-50	Present work

[a]Limit of detection. [b]Ionic liquid dispersive liquid-phase microextraction. [c]Magnetic solid-phase dispersion extraction. [d]Liquid–liquid extraction. [e]Polyamide solid-phase extraction.

CONCLUSION

The present method was settled and validated to determine eight synthetic food colorants, commercially used in gummy candy samples on the Iranian market. For this purpose, an M-DMSPE method as a simple and rapid with the low-cost procedure was applied. Zein incorporation in the adsorbent known as a biological protein is relatively inexpensive and environmental-friendly, being under the green synthesis concept. The route of the magnetic GO@zein adsorbent leads to produce a porous and interconnected structure aerogel.

The method was validated in parameters such as linearity, precision, accuracy, and recovery. The colorant concentration in the samples varied from 3.04 to 102.64 μg/mL for Brilliant Blue FCF, Tartrazine Sunset Yellow, and Allura Red. The concentration of each synthetic colorant in real samples was below the upper limit of 50 μg/mL, except for one case (Sample 9). Among the artificial colorants determined in gummy candy samples, Allura Red was found in 70% of the samples. The method developed was proved specific to determine synthetic colorants in gummy candy samples. The adsorbent amount used in our experiments was 8 mg, which was much less than that of the one used in SPE.

Conflict of interest: Declared none.

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