A Green Switchable Hydrophilic Solvent Liquid-Liquid Microextraction Coupled with Magnetic Solid-Phase Extraction for Preconcentration and Spectrophotometric Determination of Sunset Yellow Dye

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Received: June 03, 2024; Accepted: August 30, 2024
DOI: 10.17721/moca.2024.167-173

A capable, fast, and simple switchable hydrophilicity solvent-based homogenous liquid-liquid microextraction (SHS-LLME) followed by magnetic-solid phase extraction (MSPE) on highly hydrophobic linoleic acid (LA) modified Fe_3O_4 magnetic nanoparticles (MNPs) has been developed as a new approach for the extraction and preconcentration of sunset yellow dye before its spectrophotometric determination. The main parameters affecting the efficiency of extraction procedures and signal improvement were examined and optimized. Under the optimum conditions, the method was linear in the range of 10 to 100 mg L^{-1} for the analyte, with a correlation coefficient R^2 of 0.9923 and a detection limit of 5.20 mg L^{-1}.

Keywords: sunset yellow dye, Fe_3O_4 nanoparticles, switchable hydrophilicity solvent-liquid-liquid microextraction

Introduction

Food colorants enhance the visual appeal of food, conceal natural color differences, and replace colors lost during food handling. They are commonly used in baked goods, candies, beverages, and processed foods to make them visually appealing. These colorants help mask natural color variations and restore the color lost during food processing and storage, ensuring a consistent and appetizing final product for consumers. Food colorants can be classified into natural, synthetic, and inorganic [1, 2]. Due to their stability, effectiveness, and low cost, synthetic dyes are used more often than natural dyes in the food industry. However, some synthetic dyes can metabolize into hazardous components in the human body. Several studies have reported that certain synthetic dyes can have harmful effects on human health, including genotoxicity [3,4], hyperactivity [5], DNA damage [6], and allergic reactions [7]. The Sunset Yellow (E110) is an orange-red synthetic azo dye used for food coloring in the food industry. It is widely used in various food and drink products to add or increase the orange color [8]. It can be found in products including candies, soft drinks, sauces, desserts, snacks, and processed foods; excessive use of this color may cause eczema, diarrhea, allergies, anxiety, migraine, and even cancer [6]. The maximum amount of Sunset Yellow dye should not be more than 100 mg/mL in non-alcoholic drinks with fruit juices. Thus, instantaneous detection of this dye in foods and drinks is necessary [9]. Various analytical methods have been developed to determine the presence of the dye, including high-pressure liquid chromatography (HPLC) [10,11], voltammetric methods [12,13], electrochemicals [14-16], and UV spectrophotometric methods [17,18]. Due to the low concentration of the dye and the complexity of the food matrix, direct analysis of Sunset Yellow in food products is sometimes not feasible. Therefore, sample preparation is necessary before instrumental determination. Traditionally, liquid-liquid extraction (LLE) [19], cloud point extraction (CPE) [20,21], and solid-phase extraction (SPE) [22,23] have been used to separate Sunset Yellow before instrumental analysis. Recently, a new solvent called switchable hydrophilicity solvent (SHS) has received increasing attention as used in the pre-concentration step, which could be reversibly switched from hydrophobic to hydrophilic [24]. In the SHS method, the nonionic liquid converts to an ionic liquid in the presence of CO_2 or acid and then returns to its nonionic form of an alkali solution [25]. Magnetic solid-phase extraction (MSPE) is a type of dispersive solid-phase extraction. This method adds a magnetic sorbent to an aqueous sample to adsorb the target analytes. [26]. To enhance selectivity and relieve time, the combined extraction methods are very favorable; combining extraction techniques allows an effective extraction and purification process for many compounds.

In this study, switchable-hydrophilicity solvent–based liquid-liquid microextraction (SHS-LLME) has been used as the preconcentration step, followed

by magnetic solid phase extraction (MSPE) for the extraction of the Sunset Yellow dye from model solutions before its spectrophotometric determination. Experimental parameters affecting the performance of both extraction steps were optimized.

Experimental part

Apparatus

FT-IR spectra were obtained in a KBr disk on a SHIMADZU FTIR-8400S Fourier Transform Spectrometer, covering the 400-4000 cm^{-1} range. A Shimadzu UV-1650 double-beam UV/Vis spectrophotometer (Shimadzu, Japan), equipped with a 1 cm (0.5 mL) quartz cell, was used for absorbance measurements at 482 nm. A Transmission Electron Microscope Model A12AB, Germany, was used for accurate images of (Fe_3O_4) and (MNPs-LA) magnetic nanoparticles.

Standards and materials

Sunset Yellow E110 (90%) was purchased from Alfa Aesar, (Germany). All solvents, including acetonitrile (MeCN, 99.5%), methanol (MeOH, 99.9%), ethanol (EtOH, 99.9%), toluene (99%), trichloromethane ($CHCl_3$, 99%), octylamine (OCA, 99%), and N,N-dimethylcyclohexylamine (DMCHA, 99%) were purchased from Merck. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99.9%), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99.9%), linoleic acid (LA, ≥99%), triethylamine (TEA, 99.5%) and the other used chemicals were also supplied by Merck. Deionized water was used throughout the experiments.

Synthesis of linoleic acid-modified magnetic nanoparticles (MNPs-LA)

The magnetic nanoparticles (Fe_3O_4) were prepared via a simple chemical route, according to [27]. Briefly, 5.8 g of $FeCl_3 \cdot 6H_2O$ and 2.1 g of $FeCl_2 \cdot 4H_2O$ were dissolved in 100 mL of deionized water under a nitrogen atmosphere with vigorous stirring at 80 °C. Then, aqueous ammonia solution (20 mL, 25% w/w) was added. The color of the bulk solution changed from orange to black immediately. The obtained Fe_3O_4 nanoparticles were separated from the solution using an external magnet. The supernatant was removed, and the precipitate was washed sequentially with deionized water (100 mL until it turned from pH 11 to pH 7). The precipitate is dried at 50 °C until it dries completely and turns brown. Then, the solution of linoleic acid (1.0 g) was introduced dropwise at a constant rate, and the reaction proceeded under heating at 80 °C for 1h. The obtained product was further dried at 50 °C.

Recommended extraction procedure

2 mL of the standard dye solution containing 40 mg L^{-1} of analyte was transferred to a 10 mL test tube with 1.5 mL of DMCHA and 400 µL of a 6 mol L^{-1} HCl. Then, the tube was shaken manually for 20 seconds until a homogeneous phase was observed. The tube was placed at 40 °C in a water bath for 2 min. Finally, 500 µL of a 10 mol L^{-1} NaOH solution was added to the tube, and a cloudy solution appeared. The organic and aqueous phases separated completely after about 2 min. The DMCHA phase was collected using a syringe. After that, 400 µL containing 40 mg of MNPs-LA adsorbent was quickly added to the vial with the organic phase. The vial was sealed and sonicated for 2 min to facilitate the interaction of the analyte to the magnetic adsorbent. Then, the adsorbent was collected at the bottom of the vial by applying an external magnet, and the supernatant was removed. The adsorbed analyte was desorbed from the adsorbent by adding 2 mL of $CHCl_3$ for 2 min. After desorption, it was used to take UV spectra at a wavelength of 482 nm. The extraction recovery (R, %) of Sunset Yellow Dye from model solutions was calculated using the following equation :

$$\%, R = (C_{col}.V_{col} / C_o.V_{aq}) \cdot 100,$$

where C_{col}, C_o, V_{col}, and V_{aq} were the analyte concentrations in the collected organic phase, the initial analyte concentration in the aqueous solution, the volume of the collected organic phase, and the volume of the Initial aqueous solution, respectively.

Results and discussion

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy was used to identify the functional groups. Figure 1a depicts the infrared spectrum of Fe_3O_4 nanoparticles in the 400-4000 cm^{-1} wave number range. The spectrum showed the presence of a wide beam at position 3439 cm^{-1}. This beam is attributed to the substantial expansion vibrations of the (-OH) group. The beam that appeared at 1636 cm^{-1} is due to the deformation of water molecules. The beams that appeared at 638-590 cm^{-1} are due to the Fe-O bonding and the Stretching of the Fe–O bond. The infrared spectrum of the modified Fe_3O_4 with linoleic acid (MNPs-LA) is shown in Figure 1b. A wide band at 3443 cm^{-1} indicates stretching vibrations (-OH) within the carboxyl group. Additionally, vibrations of water molecules on the surface of the iron are observed in the range 3733-3898 cm^{-1}. At 1742 cm^{-1}, there is a band attributed to the stretching vibration (C=O) of the carboxylic group, and at 1647 cm^{-1}, there is a band due to the stretching vibration of the oxidized cis (C=C) groups. Within the structure of linoleic acid, bands within the range 2927-2854 cm^{-1} are due to the symmetric and asymmetric expansion vibrations of the ($-CH_2$) group, and the bands at the range 1457 cm^{-1} are due to the bending vibrations of the ($-CH_3$) and ($-CH_2$) group. The band appearing at 1166 cm^{-1} is attributed to the expansion vibrations of the (CO) group, while the band at 1101 cm^{-1} is attributed to the stretching vibrations of the (-CH) group and the deformed (-CH) group of the acid. The bands in the 573-637 cm^{-1} range are related to the Fe-O bond in the prepared compound.

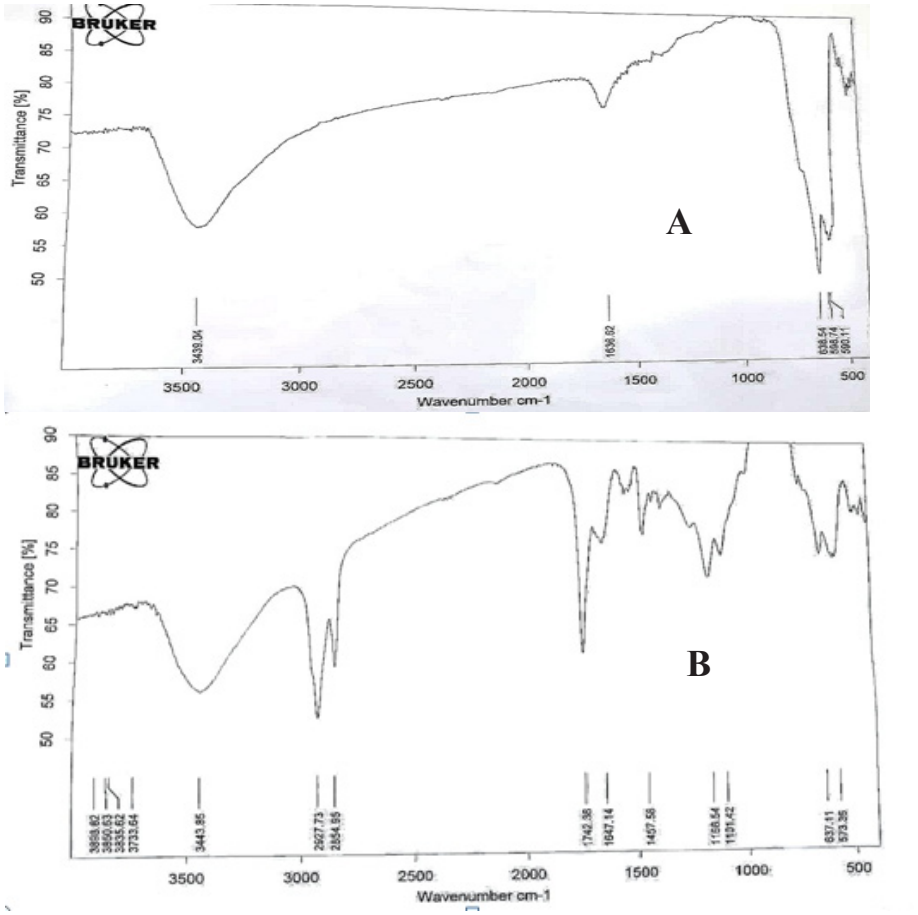

Fig. 1. FT-IR spectra of (a) Fe_3O_4 and (b) MNPs-LA.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is used to study the surface structure, particle shape, size, and distribution. Figure 2a presents an image of Fe_3O_4 nanoparticles captured using TEM. The higher magnification powers of the TEM image reveal more details about the shape of the nearly spherical Fe_3O_4 nanoparticles. Figure 2b shows an accurate image of (MNPs-LA) nanoparticles using transmission electron microscopy. The dark-colored nanoparticles represent the Fe_3O_4 NPS nucleus and are coated with dark particles representing linoleic acid. The image shows that black nanoparticles densely protect the surface of the linoleic acid, and the color is uniformly distributed. The Fe_3O_4 nanoparticles are distributed on the surface of the acid to prevent large agglomeration.

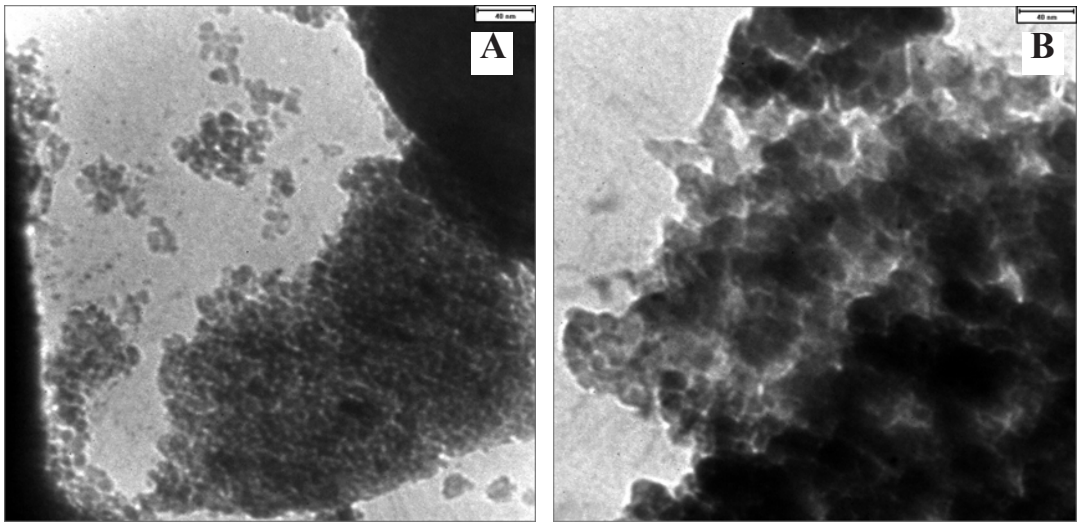

Fig. 2. TEM images of (a) Fe_3O_4 and (b) linoleic acid-modified Fe_3O_4 (MNPs-LA).

Optimization of the SHS-LLME conditions

To maximize the extraction efficiency, experimental factors influencing SHS-LLME (i.e., type and volume of SHS, HCl sodium volume, and hydroxide volume as a phase-separation trigger) were tested and optimized using the one-factor-at-a-time methodology.

Selection of the extracting solvent

The selection of the extractant is crucial for microextraction to ensure effective extraction performance. In this study, three organic solvents with switchable properties, namely N,N-dimethylcyclohexylamine (DMCHA), triethylamine (TEA), and octylamine (OCA), were examined as potential extraction solvents. All these solvents have a lower density than water. In the preliminary experiments, as shown in Figure 3a, the DMCHA solvent exhibited the highest extraction recovery for the analyte and was thus considered the most suitable extraction solvent for further experiments.

Effect of the volume of the extraction solvent

An appropriate amount of extraction solvent should be used to ensure thorough extraction without further dilution. The volume of DMCHA varied between (0.5–3.0) mL, and its effect on recovery was examined. The minimum volume providing the highest recovery was 1.5 mL of DMCHA, which was established as the optimal value for subsequent experiments (see Figure 3b for these results).

Effect of acid volume

The key to the switchable solvent microextraction is the use of acids, which serve as the molecular trigger that changes the solvent's polarity. This polarity change makes the solvent either hydrophobic or hydrophilic, allowing for the selective extraction of the analyte. We chose HCl as our acid and utilized the SHS-LLME method to determine the optimal volume. We systematically varied the volume from 100 µL to 600 µL, and the results, as shown in Figure 3c, indicated that the extraction efficiency for the sensitive yellow dye was highest at 400 µL of HCl. This suggests that 400 µL of HCl is the best volume for future extractions. Additionally, it is important to note that the analyte's likelihood of protonating increases with a higher concentration of H^+.

Effect of base volume

The key reagent in switchable solvent-based LLME is a base responsible for the extracts' phase separation. This occurs by altering the ionization state and hydrophilicity of the SHS, allowing for the dispersion and assembly of the organic phase containing the enriched analytes. It is important to optimize the amount of base (sodium hydroxide) used. The study investigated the effect of varying the 10.0 M sodium hydroxide volume in the 100–700 µL range. Figure 3d demonstrated that a volume of 500 µL is optimal, providing maximum recovery. Subsequently, this volume was used for the next extraction to maximize analyte recoveries and obtain a clear phase.

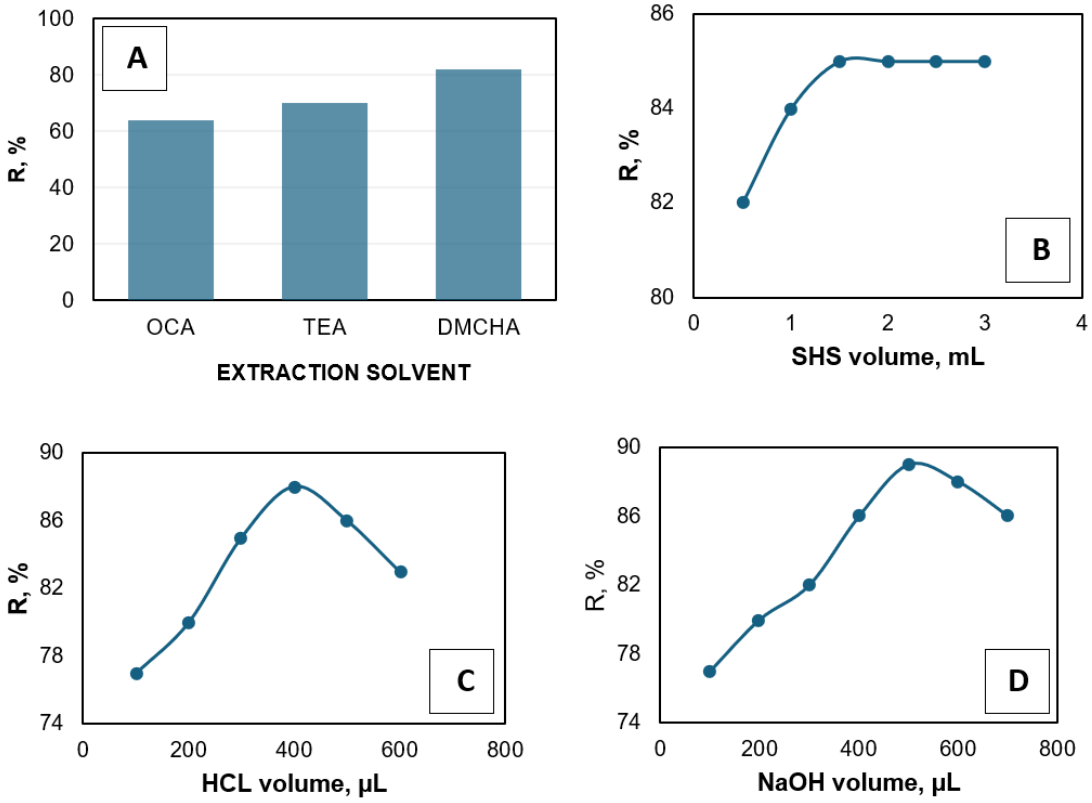

Fig. 3. Effect of (a) extraction solvent, (b) extraction solvent volume, (c) acid volume, and (d) base volume on extraction recovery (R, %) of Sunset Yellow Dye by SHS-LLME.

Optimization of the MSPE conditions

Effect of MNPS-LA amount

The quantity of adsorbent directly affects the extraction efficiency of the analyte. It ensures the complete separation of the extraction solvent containing sunset yellow dye from the solid-phase extraction process. Therefore, varying amounts of linoleic acid-modified Fe_3O_4 nanoparticles ranging from 10 mg to 60 mg were added to the sample solution. The results are summarized in Figure 4a, showing that the extraction efficiency increases with the increase in adsorbent amount up to 60 mg, after which it levels off. The high surface-to-volume ratio of nanoparticles allows for quantitative extraction using a minimal amount of adsorbent. Consequently, 40 mg was chosen for the subsequent experiments.

Desorption conditions

The best eluent for desorbing the analyte from the adsorbent was determined by testing four common organic solvents: EtOH, MeOH, MeCN, and $CHCl_3$. Fig. 4b shows that $CHCl_3$ provided the best elution of the analyte, so it was chosen as the elution solvent for the subsequent experiments. The volume of the eluent affects the method's sensitivity, determining the maximum preconcentration factor for the target analyte. Ideally, it should be as low as possible while providing a quantitative and reproducible elution of the compounds. The effect of desorbing solvent volume on dye recovery was examined in the range of 1–10 mL, and the maximum recovery was achieved with volumes higher than 1 mL. Therefore, 2 mL of $CHCl_3$ was selected for the next experiments. Additionally, the impact of desorption time on dye recovery was investigated in the range of 3–20 minutes. As shown in Fig. 4c, a desorption time of 10 minutes was found to be sufficient for complete analyte desorption.

Analytical performance of the proposed method

Model solutions of Sunset Yellow dye were prepared by spiking with working solutions and analyzed using the SHS LLME-MSPE procedure with enhanced measurement techniques. Under the best experimental conditions, the calibration curve $y = 0.0425x + 0.0297$ was linear over the 10-100 mg L^{-1} concentration range with an R^2 of 0.9989. The limit of detection (LOD) and the limit of Quantification (LOQ) were found to be 5.20 mg L^{-1} and 15.75 mg L^{-1}, respectively. (LOD = 3.3Sb/m, and LOQ = 10 Sb/m, where Sb is the standard deviation of ten replicate measurements of the blank solution, and m is the slope of the calibration curve). The preconcentration factor for the proposed method was calculated by the ratio of the sample volume of 10 mL to the final volume of 0.1 mL to be 100.

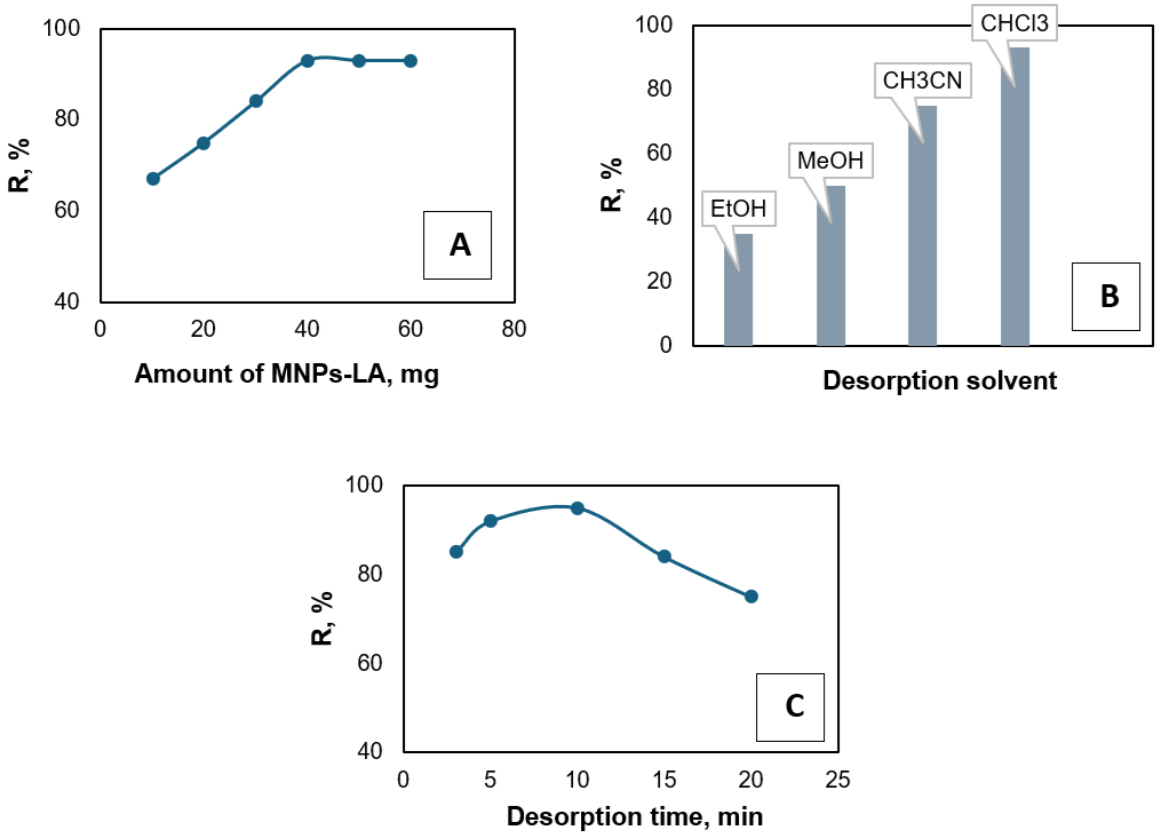

Fig. 4. Effect of (a) amount of MNPs, (b) desorption solvent, and (c) desorption time on the recovery of Sunset Yellow Dye.

Conclusion

In the present study, a combination of SHS LLME and linoleic acid-modified Fe_3O_4 MSPE was successfully applied as an effective sample pretreatment method for the trace determination of sunset yellow dye. The analytical methodology offers numerous advantages, such as ease of operation, low organic solvent consumption, high pre-concentration factor, and feasibility for large-volume samples. Furthermore, the proposed method is promising for the trace analysis of sunset yellow dye in different samples.

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