

DISPERSIVE SOLID-PHASE MICROEXTRACTION BASED ON AMINO-FUNCTIONALIZED POLYMER FOR PRECONCENTRATION OF AZO DYE FROM AQUEOUS SAMPLES

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Synthetic dyes are extensively used in the food, cosmetic, textile, and pharmaceutical industries, and their release into the environment presents serious health and ecological concerns due to their toxicity and resistance to degradation. Among them, azo dyes represent the most prevalent class and are particularly harmful due to their toxic and persistent nature. They are also linked to serious health issues, affecting organs such as the kidneys, liver, brain, and respiratory system. Therefore, the quantification and removal of azo dyes from different media is essential. In this study, the preconcentration and determination of Congo Red (CR) as a model azo dye in aqueous samples were investigated. For this purpose, dispersive solid-phase microextraction (DSPME) was employed to extract CR from aqueous samples using an amino-functionalized glycidyl methacrylate-based polymer, prior to UV-Vis spectroscopy measurements. Parameters affecting the extraction of CR, adsorption and desorption time, and desorption agent volume, were optimized by the Box-Behnken design (BBD). The optimized methodology involved 10 mL of aqueous CR solution, 50 mg of amino-functionalized polymer, and 500 μL of 0.2 mol/dm³ NaOH as the desorption agent, with sorption and desorption performed at room temperature for 4 and 6 minutes, respectively. The predicted extraction recovery of 76.07 % for CR, obtained from the polynomial model, showed good agreement with the experimental value of 75.50 %. The results confirm that the proposed DSPME procedure is a simple and fast method with strong potential for the determination of azo dyes in aqueous samples.

Keywords: glycidyl methacrylate, diethylene triamine, Congo Red, Box-Behnken design, DSPME

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INTRODUCTION

Due to their ease of use, broad color palette, and excellent stability, synthetic dyes are widely employed across industries such as textiles, leather, paper, plastics, and foodstuffs. Chemically, they consist of chromophoric compounds that interact physically or chemically with substrates, selectively absorbing specific wavelengths of light to impart color [1]. The advent of synthetic dyes enabled rapid, large-scale textile production with shorter dyeing times and enhanced dye uptake, making dyed fabrics more accessible to a broader consumer base. Beyond fiber dyeing and textile printing, they are also applied in paper and packaging coloration, as well as in the food and beverage industries [2]. Unlike natural dyes, the chemical structures of synthetic dyes can be tailored to yield a vast array of hues. However, their manufacture, use, and disposal pose significant environmental and health challenges [3].

Congo red, CR ($C_{32}H_{22}N_6Na_2O_6S_2$; $M_r = 696.68$ g/mol) is an anionic synthetic dye characterized by a complex aromatic structure [4]. Its multiple aromatic rings and azo linkage ($-N=N-$) confer resistance to biodegradation and high toxicity, as the azo bond is engineered for chemical stability [5]. CR is extensively used in textile, printing, wool, and silk industries, as well as in histological applications (e.g., amyloidosis diagnosis) and as a pH indicator [6, 7]. Owing to its persistence, CR represents a threat to human health and aquatic ecosystems: it can induce ocular and dermal irritation, gastrointestinal distress, respiratory difficulty, and is known to be carcinogenic, teratogenic, and reproductive toxic [8]. Chronic exposure may damage the liver and circulatory system and has been linked to various cancers, since CR can be metabolized to benzidine, a recognized carcinogen [9]. In aquatic environments, CR impedes light penetration and reduces gas solubility, disrupting photosynthesis and endangering aquatic life [10].

Numerous treatment methods have been developed for textile industry wastewater, which is predominantly contaminated with dyes. These include adsorption [11], membrane filtration [12], coagulation and precipitation [13], oxidation [14], photocatalysis [15], enzymatic degradation [16], and ion exchange [17]. Research is focused on developing cost-effective, locally available, and efficient adsorbents specifically designed for the removal of pollutants like CR and other azo dyes, with dispersive solid-phase microextraction recognized as a highly effective, economical, and environmentally friendly remediation strategy. Consequently, advanced hybrid materials such as amino-functionalized polymers have garnered considerable attention [18, 19].

Dispersive solid-phase microextraction (DSPME) is a modern sample-preparation technique that integrates sampling, preconcentration, and extraction in a single step, allowing the direct introduction of analytes into analytical instruments. DSPME is characterized by minimal sorbent usage and the ability to extract analytes from very small sample volumes [20]. During the development of DSPME-based methods, numerous factors influencing sample-preparation efficiency must be considered. In this context, multivariate optimization proves highly valuable. Achieving high sensitivity, accuracy, and precision with DSPME requires identifying relevant factors and experimentally controlling their individual and interactive effects. Thus, the application of formal Design of Experiments (DoE) enables a systematic, rapid, and efficient optimization workflow that significantly surpasses traditional one-factor-at-a-time (OFAT)



approaches. The choice of experimental design depends on the research objectives, the number of factors, and available resources [21].

In this work, the applicability of the DSPME method, based on an amino-functionalized glycidyl methacrylate polymer, was systematically investigated for the preconcentration and determination of CR in aqueous media. The method was optimized using the Box-Behnken experimental design to evaluate the combined effects of key operational parameters, including adsorption time, desorption time, and desorption solvent volume, in order to maximize extraction efficiency.

The advantages of the proposed method include the use of an amino-functionalized polymer as the sorbent, which enables effective analyte binding. The method requires only a small amount of sorbent, with short sorption and desorption times, contributing to overall time efficiency. Additionally, the procedure is performed under mild conditions that do not require additional energy input, and the number of experiments was minimized through the application of the DoE methodology.

MATERIALS AND METHODS

Chemicals

All the chemicals used for DSPME were of analytical grade and used as received. Congo red (CR, $C_{32}H_{22}N_6Na_2O_6S_2$), sodium hydroxide (NaOH, p.a. > 98%) were obtained from Sigma Aldrich (Saint Louis, MO, USA). All solutions were prepared with deionized water. A previously synthesized polymer, prepared via suspension polymerization and subsequently amino-functionalized with diethylenetriamine (DETA), was used as the solid phase in dispersive microextraction. The synthesis and amino-functionalization of the polymer (SGE80-deta) have been described in detail elsewhere [22].

Instrumentation

Vortex-assisted dispersive solid phase microextraction was performed employing a Vortex Stirrer (VORX-005-001, V05, Labbox Labware S.L., Spain). Statistical analysis and experimental design were conducted using Minitab (version Minitab 20, Minitab Inc, USA). Quantification of CR was carried out using UV-Vis spectroscopy (NOVEL-102S, COLOLab Experts, Polje ob Sotli, Slovenia) at an absorption wavelength (λ_{max}) of 498 nm.

Box–Behnken design (BBD)

To optimize the extraction of CR from aqueous samples, response surface methodology (RSM) based on the Box–Behnken design (BBD) was applied. The influence of key experimental variables, adsorption time (t_{ads}), desorption time (t_{des}), and desorption solvent volume (V_{des}), was systematically evaluated in order to enhance extraction efficiency prior to UV-Vis spectrophotometric analysis. Therefore, a set of 15 experiments was designed and conducted in a randomized order to prevent systematic errors. Coded factor values were used in the experimental matrix, where minimum, center, and maximum levels were assigned values of -1 , 0 , and $+1$, respectively. The values of these variables are presented in Table 1, while the matrix with coded values of the investigated factors is shown in Table 2.



Table 1. Factors affecting DSPME using the amino-functionalized polymer as the sorbent phase

Variable	-1	0	+1
Adsorption time (t_{ads}), min	2	4	6
Desorption time (t_{des}), min	2	4	6
Desorption solvent volume (V_{des}), μL	500	700	900

Table 2. BBD Matrix with Coded Values

RunOrder	t_{ads}	t_{des}	V_{des}
1	0	0	0
2	0	+1	-1
3	0	+1	+1
4	0	0	0
5	-1	-1	0
6	-1	0	+1
7	+1	0	+1
8	-1	+1	0
9	0	-1	-1
10	0	-1	+1
11	0	0	0
12	+1	-1	0
13	+1	+1	0
14	-1	0	-1
15	+1	0	-1

Dispersive solid-phase microextraction

The optimized parameters of the experimental procedure involved the use of 10 mL of aqueous CR solution (1 ppm) and 50 mg of amino-functionalized polymer as the sorbent. The sorption process was carried out at unadjusted pH (pH = 5.5) and room temperature for 4 min without any adjustment of the ionic strength, after which the sorbent was separated. For the desorption, 500 μL of sodium hydroxide solution (0.2 mol/dm³) was added, and the mixture was stirred at room temperature for 6 minutes. Following desorption, the resulting liquid phase was analyzed by UV-Vis spectroscopy at a wavelength of 498 nm to determine the CR concentration.

RESULTS AND DISCUSSION

The BBD was used to define the optimal parameter values of the DSPME method. By conducting a series of 15 experiments in three replicates, three factors, adsorption time (t_{ads}), desorption time (t_{des}), and desorption solvent volume (V_{des}), were varied at three levels. Statistical analysis of the results was performed using Minitab. The system response monitored was the extraction efficiency (ER, %) of the DSPME method, calculated according to Equation 1 [23].



$$ER, \% = \frac{C_{CR,des} V_{CR,des}}{C_{CR,aq} V_{CR,aq}} \times 100 \quad (1)$$

where $C_{CR,des}$ (mg/dm³) and $C_{CR,aq}$ (mg/dm³) denote the concentrations of CR in the desorption solvent and in the aqueous phase, respectively, while $V_{CR,aq}$ (L) and $V_{CR,des}$ (L) represent the volumes of the aqueous solution and the desorption solvent. The BBD matrix with the actual values of the examined factors, along with the experimentally obtained system response values (ER, %), is presented in Table 3.

Table 3. Experimental design for BBD with actual values of variables and the corresponding system response values

RunOrder	t_{ads} , min	t_{des} , min	V_{des} , μ L	ER, %	ER _t , %
1	4	4	700	69.16	59.05
2	4	6	500	75.50	76.07
3	4	6	900	36.40	45.91
4	4	4	700	45.24	59.05
5	2	2	700	46.92	51.41
6	2	4	900	30.80	27.27
7	6	4	900	29.19	24.99
8	2	6	700	61.32	56.73
9	4	2	500	70.30	61.63
10	4	2	900	28.74	29.07
11	4	4	700	61.47	59.05
12	6	2	700	34.05	39.53
13	6	6	700	69.09	65.49
14	2	4	500	52.80	57.91
15	6	4	500	52.70	57.07

In order to explain the correlation between the investigated factors and the system response, a second-order polynomial regression model was fitted to the experimental data, resulting in the development of the corresponding regression equation.

$$ER, \% = -7.2 + 12.2 t_{ads} - 7.9 t_{des} + 0.223 V_{des} - 2.14 t_{ads}t_{ads} + 0.70 t_{des}t_{des} - 0.000217 V_{des}V_{des} + 1.29 t_{ads}t_{des} - 0.0009 t_{ads}V_{des} + 0.0015 t_{des}V_{des}$$

The predicted values of ER, %, calculated using the developed regression equation and marked as ER_t, are presented in Table 3.

Based on these results, the lowest ER of the DSPME method using the amino-functionalized polymer as the solid phase was 28.74%, observed at a desorption solvent volume of 900 μ L, with an adsorption time of 4 minutes and a desorption time of 2 minutes. In contrast, the highest efficiency of 75.50% was achieved with a desorption solvent volume of 500 μ L, an adsorption time of 4 minutes, and a desorption time of 6 minutes. The highest extraction efficiency was achieved using 500 μ L of the desorption solvent, while a notable decrease was observed at higher volumes, such as 900 μ L. This reduction can be attributed to the dilution effect, where the analyte desorbed from the sorbent is dispersed in a larger volume, resulting in lower analyte concentration in the eluent. Consequently, the analytical signal becomes weaker, which negatively affects



the calculated recovery values. Similar observations regarding the dilution effect have been reported in other studies [23, 24]. A longer desorption period affords the analyte sufficient time to be released from the sorbent and transferred into the solvent, whereas shorter intervals often result in partial analyte retention and diminished recovery. Vortex-assisted agitation has been shown to enhance mass transfer between the sorbent and sample phases, thereby improving both adsorption and desorption efficiencies. Consequently, extending the desorption time enhances analyte transfer and improves overall extraction efficiency.

Figures 1, 2, and 3 present a three-dimensional response surface plot (a), which visually illustrates the interdependence of experimental parameters and their combined effect on the response, along with a contour plot (b) that provides a more detailed insight into the influence of individual variables and their interactions on extraction efficiency.

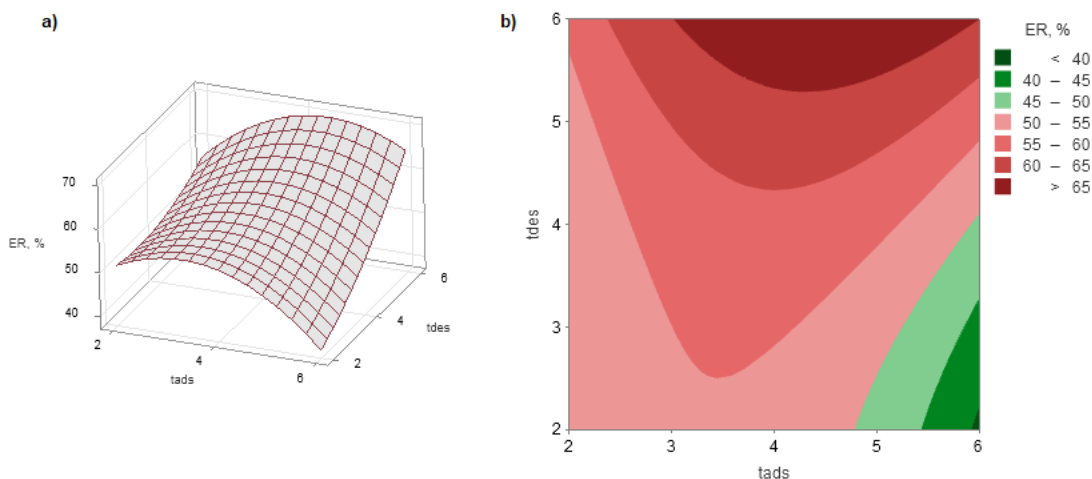


Figure 1. Effect of adsorption and desorption times on DSPME extraction efficiency at constant desorption solvent volume (700 μ l) illustrated by three-dimensional response surface plot (a) and contour plot (b)

In the three-dimensional response surface plot (Figure 1a) and the contour plot (Figure 1b), at a constant desorption-solvent volume of 700 μ L, variations in adsorption time (x-axis) and desorption time (y-axis) are shown to affect the DSPME extraction efficiency (ER, %). At short desorption intervals (2–4 min), recoveries remain moderate (approximately 50–60 %) across all adsorption durations. As the desorption time extends beyond 4 min, a marked increase in efficiency is observed: when adsorption is set to 4 min, extraction yields rise sharply, reaching their maximum at a desorption time of 6 min (> 65 %). Further prolongation of desorption or deviation from the 4 min adsorption interval affords no additional benefit and may even slightly reduce recoveries, indicating that equilibrium has been achieved. These results clearly identify 4 min of adsorption combined with 6 min of desorption as the optimal conditions for maximizing DSPME performance.



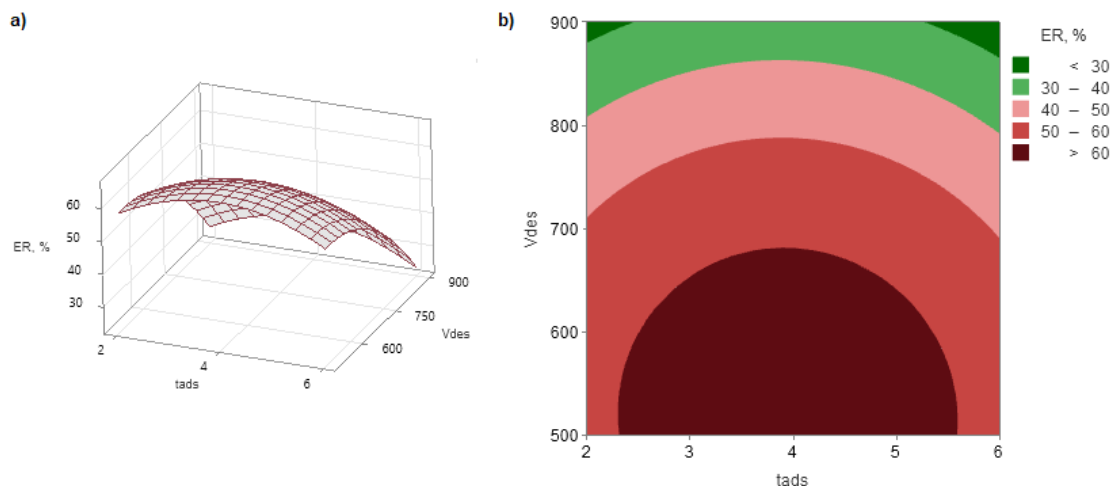


Figure 2. Response surface and contour plots showing the effect of desorption solvent volume and adsorption time on DSPME extraction efficiency at constant desorption time (4 min)

Figure 2 depicts a three-dimensional response surface (a) and contour plots (b) of DSPME extraction efficiency (ER, %) as a function of desorption solvent volume (500–900 μ L, y-axis) and adsorption time (2–6 min, x-axis), with desorption time fixed at 4 min. The highest efficiencies (ER > 60 %) are centered precisely at 500 μ L and an adsorption time of 4 min, identifying this point as the optimal operating condition. Deviations from 500 μ L, either increasing the solvent volume above 500 μ L or decreasing it below, result in a steady decline in recovery, passing through the 50–60 % and 40–50 % regions down to less than 40 % at the extremes. Similarly, adsorption times shorter or longer than 4 min yield lower efficiencies. These results confirm that a desorption solvent volume of 500 μ L combined with a 4 min adsorption is optimal for achieving maximum DSPME performance.

Figure 3 presents a three-dimensional response surface plot (a) and a contour plot (b) of DSPME extraction efficiency (R, %) as a function of desorption time (2–6 min, x-axis) and desorption solvent volume (500–900 μ L, y-axis), with the adsorption time held constant at 4 min. The darkest region (> 70 %) occurs at a desorption time of 6 min and a solvent volume of approximately 500 μ L, indicating the highest extraction yield. Moving away from this optimal point, either by decreasing the solvent volume below or increasing it above 500 μ L, or by shortening the desorption time below 6 min, leads to a gradual decrease in efficiency, passing through the 60–70 % and 50–60 %, respectively, contours and falling below 40 % at the extreme parameter values. These results clearly confirm that a 6-minute desorption interval combined with a 500 μ L eluent volume represents the optimal conditions for maximizing DSPME performance.



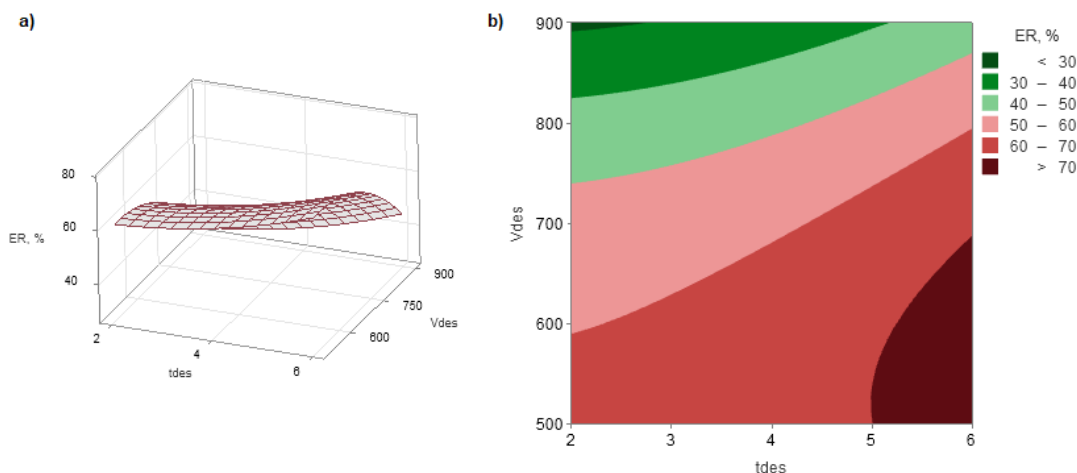


Figure 3. Response surface and contour plots showing the effect of desorption time and solvent volume on DSPME extraction efficiency at constant adsorption time (4 min)

CONCLUSIONS

In the context of modern, industry-driven lifestyles, the textile sector ranks among the fastest-growing industries and plays a pivotal role in a country's economic development. Simultaneously, it invariably exacerbates anthropogenic impacts on the Earth's biosphere by consuming vast quantities of water and generating large volumes of colored wastewater due to the dyes and pigments employed during the dyeing process. CR, an anionic azo dye, poses significant challenges in the dye industry owing to its complex chemical structure. Effective treatment of industrial effluents-often contaminated by the excessive use of CR-remains one of the most pressing and enduring ecological concerns. Therefore, the development of efficient, rapid, and environmentally benign analytical methods for the detection and removal of such pollutants is of paramount importance. In this study, the DSPME procedure utilizing an amino-functionalized glycidyl methacrylate-based polymer was successfully optimized for the selective extraction of CR from aqueous samples. The application of BBD enabled the identification of optimal operational parameters, specifically, 4 minutes of adsorption, 6 minutes of desorption, and 500 μL of NaOH as the desorption agent, yielding a high extraction recovery of over 75%. These results demonstrate that the proposed method is not only cost-effective and straightforward but also highly suitable for the monitoring and treatment of dye-contaminated water in environmental analysis.

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Izvod

DISPERZIVNA MIKROEKSTRAKCIJA NA AMINO-FUNKCIONALIZOVANOM POLIMERU KAO ČVRSTOJ FAZI ZA PREKONCENTRACIJU AZO BOJE IZ VODENIH RASTVORA

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Sintetičke boje se široko primenjuju u prehrambenoj, kozmetičkoj, tekstilnoj i farmaceutskoj industriji, a njihovo dospevanje u životnu sredinu predstavlja ozbiljan ekološki i zdravstveni problem zbog njihove toksičnosti i hemijske postojanosti. Među njima, azo boje čine najzastupljeniju klasu i naročito su štetne usled svoje toksične i perzistentne prirode. Dodatno, povezane su sa ozbiljnim zdravstvenim problemima, koji mogu zahvatiti bubrege, jetru, mozak i respiratorni sistem. Stoga je kvantifikacija i uklanjanje azo boja iz različitih medijuma od ključne važnosti. U ovom radu ispituje se prekoncentracija i određivanje azo boje Kongo crveno (CR) kao model supstance u vodenim rastvorima. U tu svrhu primenjena je disperzivna mikroekstrakcija na čvrstoj fazi (DSPME) za izdvajanje CR iz vodenih uzoraka pomoću amino-funkcionalizovanog polimera na bazi glicidil-metakrilata, pre merenja na UV-Vis spektrofotometru. Parametri koji utiču na proces ekstrakcije CR, vreme adsorpcije i desorpcije, kao i zapremina agensa za desorpciju, optimizovani su korišćenjem Box-Benken dizajna (BBD). Optimizovani uslovi obuhvatali su 10 mL vodenog rastvora CR, 50 mg amino-funkcionalizovanog polimera i 500 μ L rastvora NaOH rastvora (0,2 mol/dm³) kao agensa za desorpciju, pri čemu su adsorpcija i desorpcija sprovedene na sobnoj temperaturi tokom 4, odnosno 6 minuta. Predviđeni prinos ekstrakcije CR izračunat pomoću polinomskog modela iznosio je 76,07 %, što je pokazalo dobru saglasnost sa eksperimentalno dobijenim rezultatom od 75,50 %. Dobijeni rezultati potvrđuju da je predložena DSPME procedura jednostavna i brza metoda sa značajnim potencijalom za određivanje azo boja u vodenim rastvorima.

Ključne reči: glicidil metakrilat, dietilentriamin, Kongo crveno, Box-Behnken dizajn, DSPME

