Optimization of Switchable Hydrophilicity Solvent-Based Liquid-Liquid Microextraction for Tartrazine Dye Separation and Preconcentration using Central Composite Design and Response Surface Methodology

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Switchable hydrophilicity solvent liquid-liquid microextraction (SHS-LLME) was employed as a sample pretreatment method to enrich and pre-concentrate the dye Tartrazine (Tz) before its quantification using UV-visible spectrophotometry. N-methyl dipropyl amine (MDPA) was used as the extraction solvent. The key parameters of the SHS-LLME process were optimized using central composite design (CCD) and response surface methodology (RSM) to evaluate the interactive effects of the three most critical operational variables. The interactions between the MDPA, HCl, and NaOH volume on dye absorption were studied. The LOD and LOQ (n = 7) were calculated as 0.23 µg/mL and 0.692 µg/mL, respectively. The method showed a relative standard deviation (RSD) of 1.71% for 10 replicate measurements. Enrichment and preconcentration factors were determined to be 63 and 33.33, respectively, demonstrating the method's effectiveness in enhancing Tartrazine detection.

Keywords: tartrazine (Tz), central composite design (CCD), response surface methodology (RSM), switchable hydrophilicity solvent (SHS), liquid-liquid microextraction (LLME)

Introduction

In recent years, concerns about the health risks associated with synthetic dyes, particularly in food, have grown significantly. Azo dyes, the most common class of synthetic dyes, are widely used in various applications, including as color additives in food [1,2], paints [3], cosmetics [4], as well as in biomedical and optoelectronics industries [5,6]. Among these, Tartrazine (Tz), a widely used azo dye, is an anionic molecule containing a benzene ring and is commonly used as a colorant in pharmaceuticals, textiles, cosmetics, and food industries [8]. However, Tartrazine is considered one of the most harmful azo dyes to human health, especially when used in high concentrations. It has been associated with adverse health effects such as migraines, eczema, asthma, lupus, and even thyroid cancer [9,10]. Due to these risks, strict monitoring of its content, particularly of food products, is essential.

Several analytical methods have been developed to detect azo dyes [11-13], particularly Tartrazine, in various samples. Techniques such as voltammetry [14], electrochemical methods [15,16], UV-visible spectrophotometry [17,18], and high-performance liquid chromatography (HPLC) [19,20] have been used. However, these methods often require expensive equipment and skilled operators, making them less accessible to many laboratories. Furthermore, because of the low levels of Tartrazine in samples, direct analysis using these instruments is often inadequate, requiring an additional step to enrich the sample. Commonly used methods for this purpose include solid-phase extraction (SPE) [21], liquid-liquid extraction (LLE) [22], and cloud point extraction (CPE) [23].

A novel method known as "switchable hydrophilicity solvent-based liquid-liquid microextraction (SHS-LLME)" has been introduced as an effective preconcentration technique [24-27]. Switchable hydrophilicity solvents (SHS), also called "smart solvents," such as tertiary amines, are soluble in water in the presence of acid or carbon dioxide. When tertiary amines react with these compounds, an acid-base reaction occurs, producing a protonated polar SHS that becomes soluble in water. Upon the addition of a strong base like NaOH, the reaction is reversed, restoring the original non-polar form of the SHS. This property enables the selective enrichment of hydrophobic organic analytes [28]. The response surface methodology (RSM) is widely used to optimize such extraction processes. RSM is a powerful statistical and mathematical tool that allows researchers to explore the relationships between variables and responses and analyze their interactions. It provides a mathematical model to assess the effects of independent variables, and by utilizing analysis of

variance (ANOVA), it creates a data-driven model to guide experimental optimization [29].

In this study, central composite design (CCD) and response surface methodology (RSM) were employed to optimize the switchable-hydrophilicity solvent-based liquid-liquid microextraction (SHS-LLME) method for the extraction and enrichment of Tartrazine dye before its spectrophotometric determination.

Experimental part

Apparatus

For absorbance measurements at 430 nm, a Shimadzu UV-1650 double-beam UV/Vis spectrophotometer (Shimadzu, Japan) equipped with a 1 cm path length quartz cell (0.5 mL capacity) was used. The experimental process also utilized an Ultrasonic Bath LUC-410 (Labtech, Korea) and a Centrifuge EBA-20 (Hettich, Germany).

Standards and materials

A Tartrazine (Tz) standard (98.0%) was purchased from India company. The solvents, including ethanol (EtOH, 99.9%) and N,N-dimethylcyclohexylamine (DMCHA, 99%), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Additionally, triethylamine (TEA, 99.5%), N-methyl dipropylamine (MDPA, 98%), and other chemicals used in the study were supplied by Merck.

Standard Microextraction Procedure

In a 10 mL conical-bottom centrifuge tube, 2 mL of a Tartrazine dye solution (2 μg/mL) was transferred. Then, 0.5 mL of N-methyl dipropylamine (MDPA) was added as the extracting phase. Subsequently, 0.6 mL of a 6 mol/L HCl solution was added to the tube, which was manually shaken for 20 seconds until a homogeneous phase was observed. The tube was then placed in a 40°C water bath for 2 minutes. After that, 0.5 mL of 10 mol/L NaOH solution was added, and ultrasonication was performed for 40 seconds. At this point, a cloudy solution formed. The fine droplets were separated by centrifugation at 3500 rpm for 3 minutes. The aqueous phase was carefully discarded using a microsyringe, while the remaining organic phase was diluted with 3 mL of ethanol. Finally, the absorbance was measured using a UV-visible spectrophotometer at a wavelength of 430 nm.

Optimization Strategy

A central composite design (CCD) using response surface methodology (RSM) was used to optimize the key factors influencing the SHS-LLME procedure. After initial trials, it was found that three parameters — MDPA volume, HCl volume, and NaOH volume — were the independent factors affecting the extraction of Tartrazine. The dye's absorbance was chosen as the response for optimization. A three-factor, three-level CCD was created to optimize these parameters using Minitab version 19.0.1 (trial) for the analysis. In the CCD design, MDPA, HCl, and NaOH volumes were denoted as A, B, and C, respectively. The levels, codes, and units of the optimized factors can be found in Table S1. The three independent variables — MDPA volume (A), HCl volume (B), and NaOH volume (C) were tested at three different levels to understand their impact on the extraction efficiency of Tartrazine. The objective was to maximize the absorbance response.

Results and Discussion

Optimization of SHS-LLME Factors

The most significant parameters affecting the SHS-LLME process, including the type and volume of the extraction solvent, extraction time, acid volume, and base volume (as a switching-off trigger), were studied and optimized. The impact of each parameter on the dye's absorbance was evaluated using the one-factor-at-a-time (OFAT) approach. This method allowed for the systematic assessment of each factor's influence on the extraction efficiency, helping to identify the optimal conditions for maximizing dye absorbance.

Optimization of the Type and Volume of SHS

The selection of an appropriate switchable hydrophilicity solvent (SHS) is crucial and must meet specific criteria: (a) it should exhibit dual hydrophobic and hydrophilic properties that can be switched by the addition or removal of CO_2 or an acid; (b) to form a stable two-phase system, the hydrophobic solvent should be insoluble in water in its neutral form, and when CO_2 or an acid is introduced as a trigger, it must convert into a protonated, water-miscible form; and (c) the solvent should have a strong extraction affinity for the analytes. Tertiary amines, such as triethylamine (TEA), N,N-dimethylcyclohexylamine (DMCHA), and N-methyl dipropylamine (MDPA), are commonly used as solvents in SHS-LLME due to their ability to create a large contact surface between the analyte solution and the solvent in a homogeneous medium. Among these, MDPA has been observed to yield the highest absorbance for the analyte (as shown in Fig. 1a), likely due to its greater hydrophobicity. The volume of the SHS solvent can significantly influence the volume of the resulting SHS phase and the recovery of the dye. Different volumes of MDPA (ranging from 0.1 to 0.9 mL) were tested. As shown in Fig. 1b, the optimal volume was found to be 0.5 mL. Beyond this volume, the absorbance decreased, likely due to an increase in the supernatant volume, which reduced the concentration of the extracted dye in the SHS phase.

Effect of Acid Volume

Acids act as molecular triggers that alter the polarity of the solvent, allowing it to selectively extract the analyte by transitioning between hydrophobic and hydrophilic states. To determine the optimal acid dosage, HCl was chosen as the acid, and its volume was systematically varied from 0.1 to 0.9 mL while using the SHS-LLME method. The volume of the extraction solvent was kept constant at the previously optimized level. As shown in Fig. 2a, the ideal HCl volume for maximizing extraction efficiency was found to be 0.6 mL, making it the optimal choice for future extractions.

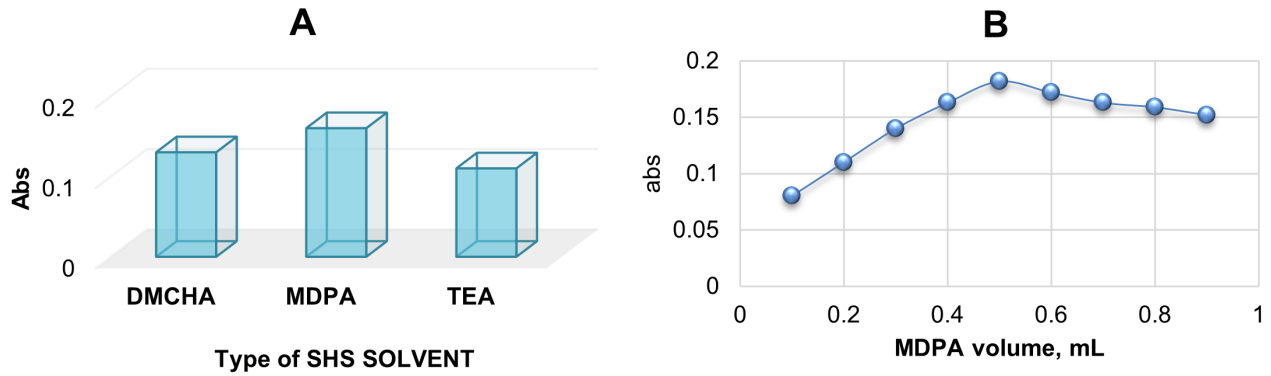

Fig. 1. Effect of a) SHS Type and b) MDPA volume on the Absorbance of Tartrazine Dye.

Effect of Extraction Time

In the SHS-LLME method, extraction time is defined as the interval between the addition of the base (NaOH) and the phase separation. The influence of extraction time was studied over a range of 10–60 seconds. As shown in Fig. 2b, the extraction efficiency increased up to 40 seconds, after which it remained constant. In the SHS-LLME process, equilibrium is reached quickly due to the homogeneous nature of the extraction. Based on these results, 40 seconds was determined to be the optimal extraction time.

Optimization of NaOH Volume

In the SHS-LLME method, phase separation is only achievable when a high concentration of 10 M sodium hydroxide (NaOH) is added to the homogeneous extraction system. This triggers the inactivation of the tertiary amine, inducing phase separation. Different volumes of 10 M NaOH, ranging from 0.2 to 0.8 mL, were evaluated. As shown in Fig. 2c, dye absorbance increased with the addition of NaOH up to 0.6 mL, after which it remained constant. Therefore, 0.6 mL was determined to be the optimal volume for further experiments.

Experimental design

In previous experiments, a One-Factor-At-A-Time (OFAT) approach was used to define the factor ranges for subsequent application of Response Surface Methodology (RSM). A Central Composite Design (CCD) was then developed to explore the interactions between the extraction factors and optimize the

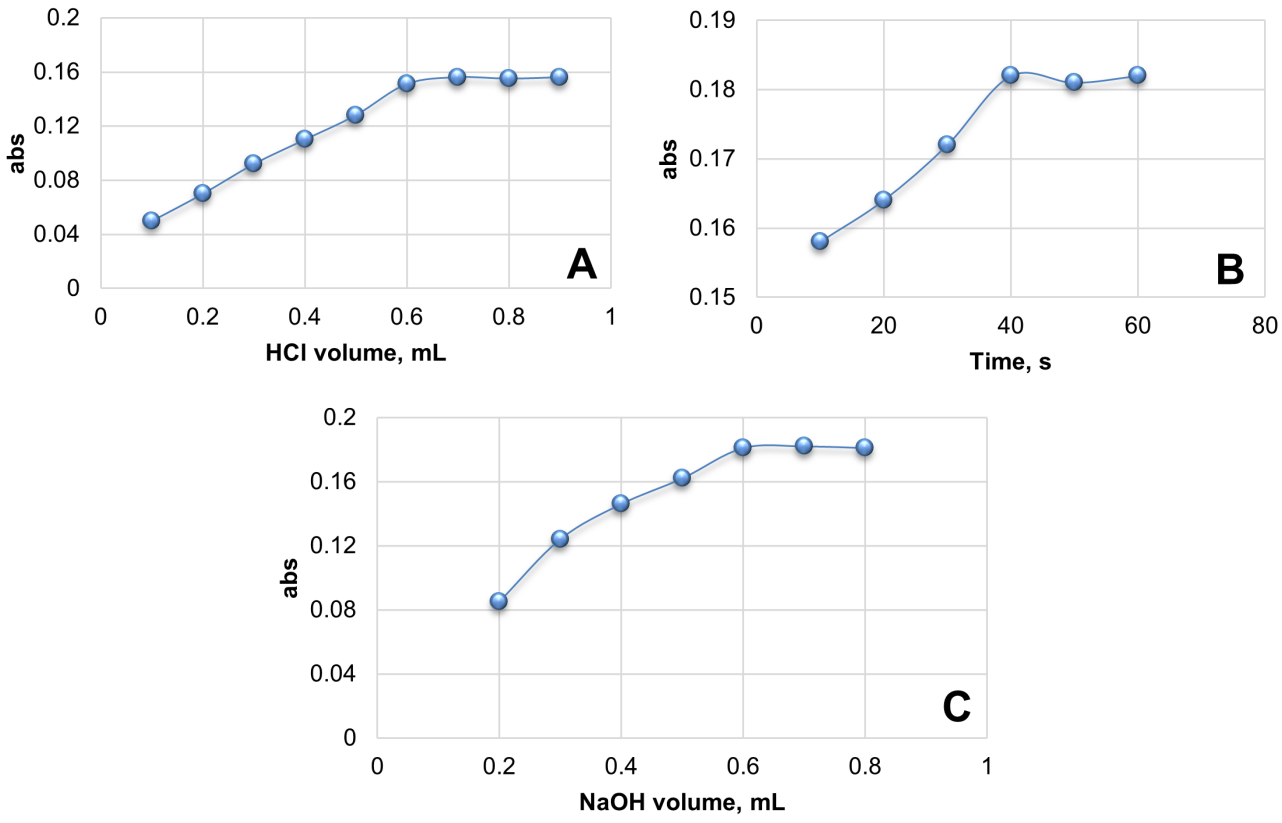

Fig. 2. Effect of (a) Extraction Time, (b) HCl Volume, and (c) NaOH Volume on the Absorbance of Tartrazine Dye.

conditions for maximizing tartrazine dye extraction efficiency. The study utilized a bivariate cubic model with three independent factors: MDPA volume (A), HCl volume (B), and NaOH volume (C), each evaluated at three levels. Six replicates were performed at the central point to ensure robustness.

For the CCD, the total number of experiments (N) required for three factors at three levels was 20. This was calculated as 2^k (for the factorial points, with k=3, so 2^3=8), plus $2 \cdot k$ (giving 6 axial points), and 6 replications at the central point. Thus, the design included 8 factorial points, 6 axial points, and 6 center point replicates, leading to a total of 20 experiments.

The experimental design used for optimization and the corresponding responses for the 20 experiments are presented in Table S2.

The responses corresponding to the coded values of the three distinct variables were subjected to quadratic regression modeling, which aligned with the Response Surface Methodology (RSM) results. The predicted absorbance (abs) for the dye was determined using the following equation:

abs=0.1079+0.0842A+0.0390B+0.1953C−0.0368A2−0.1458B2−0.0948C2+0.0422AB+0.0387BC−0.0875AC

The fitted quadratic model, as presented in Table S3, was statistically significant according to ANOVA under a 95% confidence level. A smaller p-value (below 0.05) indicates greater significance. The final model yielded a p-value of 0.001, indicating that the average pore size inside the fold differs significantly from zero to the 95% confidence level. The terms ranked with the smallest p-values, which are considered significant, include the HCl volume (B), NaOH volume (C), the interaction between MDPA volume and HCl volume (AB), the interaction between MDPA volume and NaOH volume (AC), the quadratic term for HCl volume (B^2), and the quadratic term for NaOH volume (C^2).

Conversely, the p-values for MDPA volume (A), the interaction between MDPA and NaOH volumes (AC), and the quadratic term for MDPA volume (A^2) were high, indicating a low probability of significance. Therefore, these parameters were excluded from the final quadratic model. The significant effects of the coded variables on the relationship between the predicted response and the independent experimental factors were as follows:

abs=0.1079+0.0390B+0.1953C−0.1458B2−0.0948C2+0.0422AB+0.0387BC

The model's determination coefficient (R^2) revealed that the independent variables explain 98.27% of the variation in dye absorbance, leaving only 1.73% unexplained. The high value of the adjusted determination coefficient (96.98%) further supports the significance and reliability of the developed model.

Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots were generated to visualize the effects of interactions between variables on dye absorbance. Figure 3 shows the relationship between HCl volume and NaOH volume on dye absorbance, while the third variable, MDPA volume, was held at its center level. The results clearly indicate that absorbance depends on all the experimental variables studied. Additionally, the plots highlight significant interactions between the variables.

Finally, Table 4 shows the optimal conditions for maximizing dye absorbance, derived from the model, for further examination.

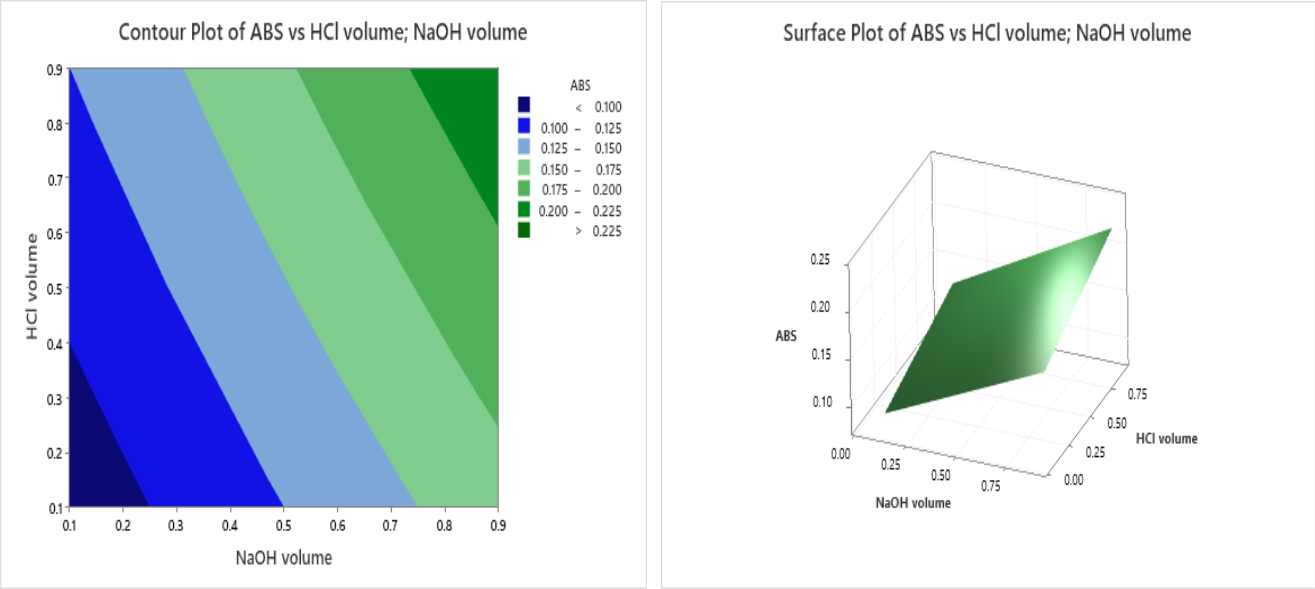

Fig. 3. Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots showing the effect of HCl volume and NaOH volume on dye absorbance, with MDPA volume held constant at the center level.

Table 4. Optimum conditions attained by Response Surface Methodology (RSM).

Variable	Optimum Range	Selected Value
HCl volume	0.6 - 0.9	0.7
NaOH volume	0.7 - 0.9	0.75

Analytical Performance

Under the optimized conditions determined in the previous steps, the analytical performance of the method was evaluated. The calibration curve, described by the equation y=0.0153x+0.0025, was linear over the concentration range of 0.5–10 μg/mL, with a high correlation coefficient (R^2=0.9989). The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.23 μg/mL and 0.692 μg/mL, respectively. These values were determined using the formulas LOD=3.3Sb/m and LOQ=10Sb/m, where Sb is the standard deviation of seven replicate measurements of the blank solution, and m is the slope of the calibration curve.

The relative standard deviation (RSD) for ten replicate measurements of a 2.0 μg/mL solution was 1.71%, indicating good precision. The enrichment factor, defined as the ratio of the slope of the calibration curve with and without the SHS-LLME technique, was found to be 63. The preconcentration factor for the method was calculated as the ratio of the sample volume (10 mL) to the final volume (0.3 mL), yielding a value of 33.33.

Conclusion

A simple solvent microextraction technique utilizing switchable hydrophilicity solvent liquid-liquid microextraction (SHS-LLME), combined with UV-Vis spectrophotometry, was successfully applied for the separation and enrichment of tartrazine dye. Various parameters were systematically studied, including the type and volume of the extraction solvent, HCl volume, extraction time, and NaOH volume. Subsequently, the most significant factors were optimized using a Central Composite Design (CCD). By applying Response Surface Methodology (RSM) to the CCD data, the optimal extraction conditions for tartrazine dye separation were accurately determined. RSM revealed that this enrichment step requires careful experimental design to identify the precise relationship between extraction efficiency and influential factors, demonstrating the effectiveness of the method in optimizing dye separation.

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