

Preconcentration of Sudan IV dye using β -Cyclodextrin Butanediol Diglycidyl Ether Polymer as the Solid Phase Extractant

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ABSTRACT

A solid phase extraction method for the preconcentration of Sudan IV dye at the trace level using newly synthesized β -cyclodextrin butanediol diglycidyl ether polymer (β -CDP). The residual concentration of the dye solute was determined by spectrophotometric method. Various parameters such as effect of pH, sample volume, shaking time, amount of adsorbent for the % uptake of the Sudan IV dye have been optimized. The method has been used for the determination of Sudan IV dye in different food samples.

Keywords: β -Cyclodextrin, β -Cyclodextrin 1,4-butanediol diglycidyl ether polymer, Sudan IV dye, preconcentration, Spectrophotometry.

I. INTRODUCTION

Dyes play a significant role in food industry as food quality and flavor are closely associated with color of food. Dyes have been the subject of much interest in recent years because of increasingly strict restrictions on the organic content of industrial effluents. Food colorant or food dyes are both used in commercial food production and in domestic cooking. Sudan IV is a synthetic diazo dye used for the staining of lipids, triglycerides and lipoproteins on frozen paraffin sections. Sudan IV is one of the dyes used for Sudan staining. For staining purposes Sudan IV can be made up in propylene glycol^[1]. It is typically used in many industrial applications including solvents, oils, fats, waxes, plastics, printing inks, and floor polishes^[2-3]. The main reason for the widespread usage is its colorfastness and low price. However, azo-colorants are biologically active through their metabolites^[4-5] and have been associated with increased occurrence of bladder cancer in textile and leather dyers, painters, and hairdressers^[6-7]. Due to their potential carcinogenicity, many countries have banned the use of most azo dyes at any level in products for human consumption^[8]. Different techniques are used for the separation and preconcentration of dyes in the solution. These includes GC^[9], LC^[10-11], CE^[12] and HPLC^[13]. Some methods are

very costly and required the expert hands. So, spectrophotometry is widely used for the determination of dyes due to its higher sensitivity, low cost, low interference level and its excellent detection limits. So, Sudan IV dye has been determined by spectrophotometric methods after preconcentration using β -CDP in food samples.

Supramolecular complexes with β -cyclodextrin has been a very active research field in the past few years^[14-15]. β -cyclodextrin (β -CD) is a very stable oligosaccharide that is composed of seven glucose units linked with each other by α -(1,4)-glycosidic linkage. It can form supramolecular complexes with several organic compounds by incorporating them into their hydrophobic cavities. Two or more β -cyclodextrin covalently linked with each other are known as polymers. These β -cyclodextrin polymer have been used for the preconcentration of various analytes^[16-19]. In the present work, β -cyclodextrin butanediol diglycidyl ether polymer (β -CDP) has been used as a solid support for the preconcentration of Sudan IV dye.

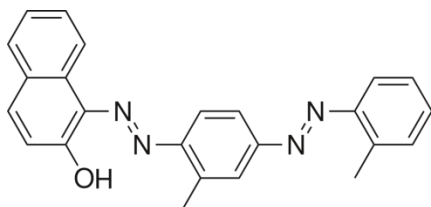


Figure 1. Structure of 1-[2-methyl-4-(2-methylphenyldiazenyl)phenyl] azonaphthalen-2-ol (Sudan IV Dye)

II. Experimental

A Shimadzu UV-1800 spectrophotometer (Shimadzu Ltd., Japan) equipped with the matched 10 mm quartz cells was used to measure absorbance. Digital century pH-meter Cp - 901 with a combined glass electrode was used to carry out pH measurements. A thermostatic shaking water bath (Perfit India Ltd.) was used to carry out all the inclusive procedures.

Reagents

All chemicals used were of AnalR grade unless otherwise stated. Double distilled water was used throughout the experiment. Brilliant green dye solution was prepared by dissolving 0.482 g in 100 mL of double distilled water to give 0.01 M standard stock solution and further diluted as and when required.

The synthesis of β -Cyclodextrin polymer (β -CDP) from β -Cyclodextrin and crosslinker 1,4-Butanediol diglycidyl ether involves the abstraction of hydrogen ion from free primary and secondary hydroxyl groups to form oxy anions (I). The resulting oxy anion then attacks on to the epoxide ring of the cross-linker resulting in the generation of a new oxy anion which then abstracts the hydrogen ion (H^+) from the medium. This reaction goes on and results in the formation of cross-linked β -cyclodextrin polymer and dried at 100 °C and then stored at room temperature in the desiccators for further use.

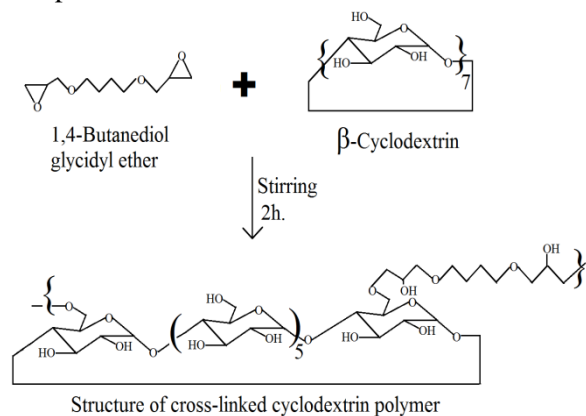


Figure 2. Schematic representation of the synthesis of β -cyclodextrin polymer.

The synthesized polymers were subjected to SEM analysis to study their morphological aspects. SEM Scans of the polymers were carried out at Sophisticated Instrumentation centre (SIC), Punjabi University, Patiala on JEOL (Tokyo, JAPAN) JSM-6510/LV-SEM Instrument. For the above experiment 1mg. of the sample was mounted on a glass slide and subjected to scan at an acceleration voltage of 10Kv. The SEM images were taken at five different magnifications from X200, X1000, X2500, X3000 and X5000. From the SEM micrographs it is obvious that the structure of β -CD is rock like and β -CDP is relatively fibrous which shows that cross-linking has taken place.

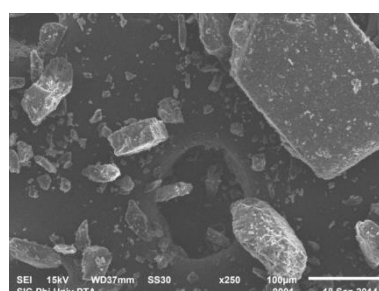


Figure 3. SEM scan of β -CD at X200

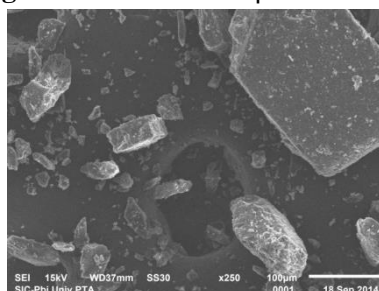


Figure 4. SEM scan of β -CD at X1000

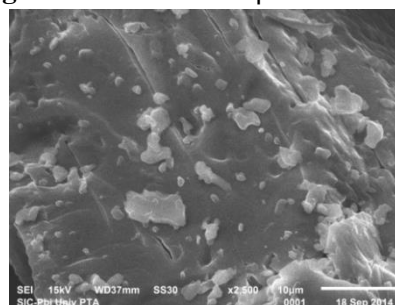


Figure 5. SEM Scan of β -CD at X2500

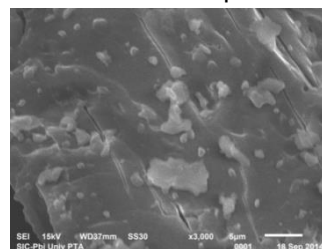


Figure 6. SEM Scan of β -CD at X3000

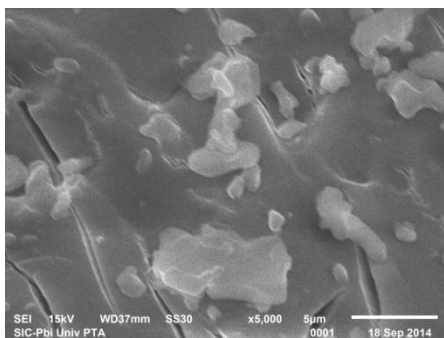


Figure 7. SEM Scan of β -CD at X5000

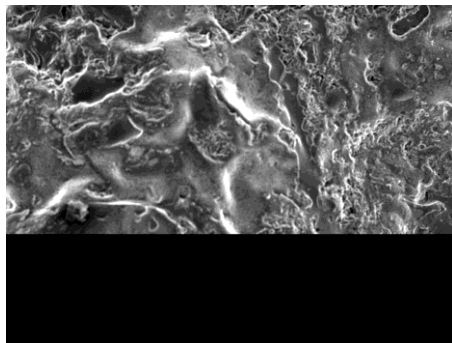


Figure 8. SEM Scan of β -CDP at X200

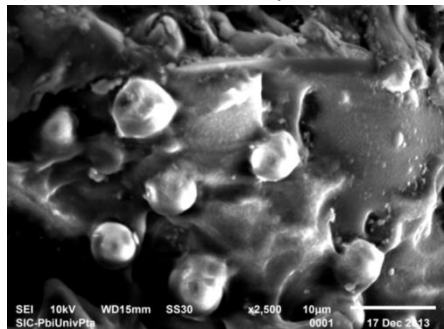


Figure 9. SEM Scan of β -CDP at X2500

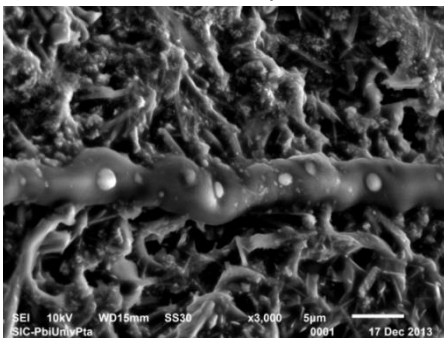


Figure 10. SEM Scan of β -CDP at X3000

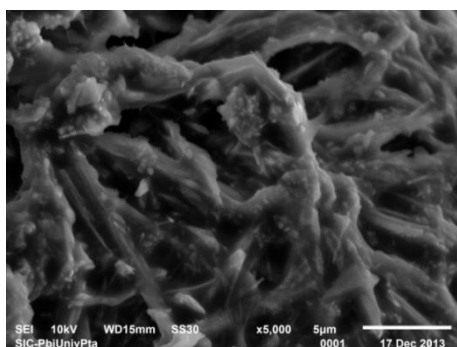


Figure 11. SEM Scan of β -CDP at X5000

Fourier Transform Infra-red (FT-IR) Analysis

The reactant β -Cyclodextrin (β -CD), the synthesized β -Cyclodextrin polymer (β -CDP) were subjected to IR analysis on a Pro resolution FTIR system as KBr pellets in the range $4000-400\text{ cm}^{-1}$ region. From FT-IR (KBr) of β -CD and β -CDP it can be seen that: for β -CD, $3600-3000\text{ cm}^{-1}$ (OH), 2900 cm^{-1} (CH); while for β -CDP a sharp band at 3400 cm^{-1} (OH) is obtained

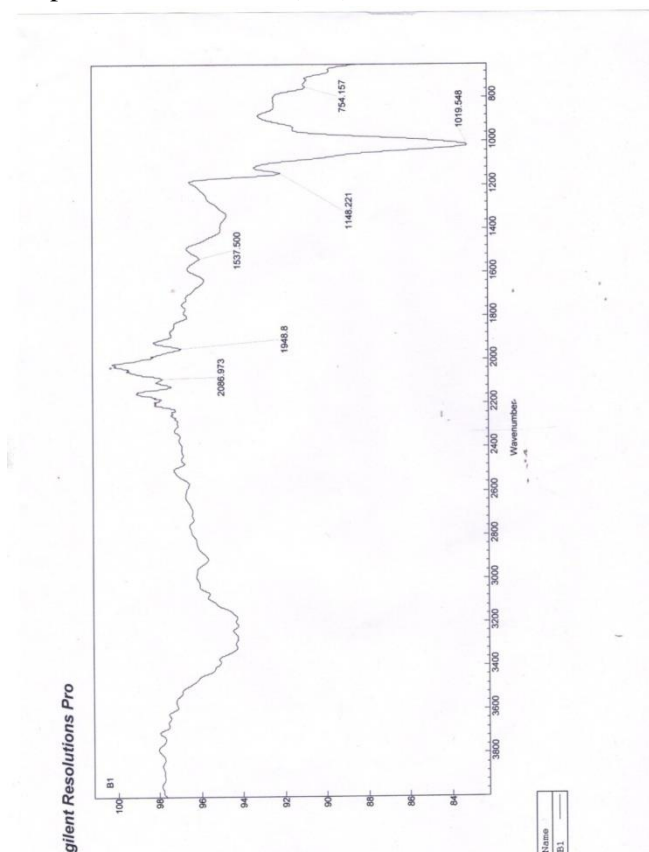


Figure 12. FT-IR Spectrum of native β -Cyclodextrin

III. Results and Discussion

Optimization of various parameters

Effect of pH

The formation of inclusion complex of the dye with the polymer depends on the pH of sample solution. The pH of the solution was adjusted in the range of (1.0 to 7.0) using different buffer solutions and then the preconcentration procedure as described was applied (Fig. 15), quantitative uptake ($\geq 95\%$) was obtained at pH 2.0. Therefore, the working pH 2.0 was chosen for the subsequent studies

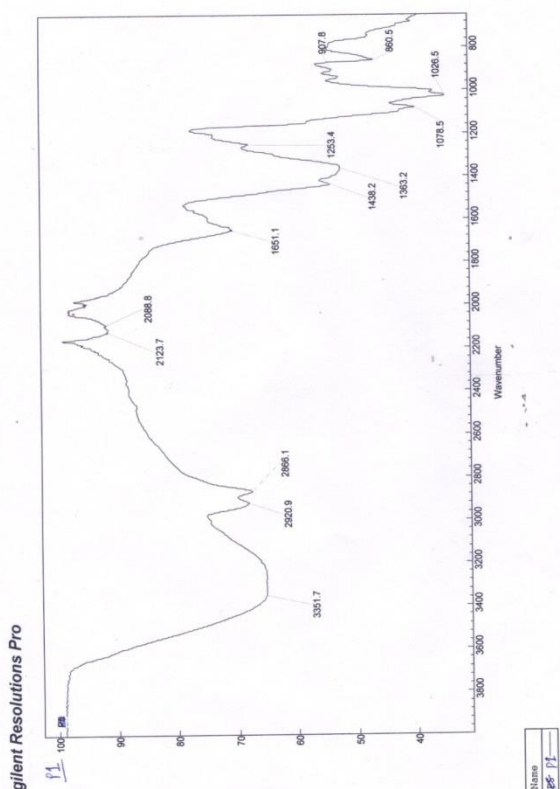


Figure 13. FT-IR Spectrum of cross-linked β -Cyclodextrin polymer (β -CDP)

Buffer solution in the pH range of 2.0-3.5 were made by mixing equimolar solutions of hydrochloric acid/Sodium Acetate and buffer solutions in the pH range of 4.0-6.5 were made by mixing equimolar solutions of sodium acetate and acetic acid solutions in the different proportions While those in the pH range of 7.0-11.0 were made by mixing equimolar solutions of ammonia and ammonium chloride. The glass wares were washed with chromic acid and soaked in 5% nitric acid and then cleaned with double distilled water before use and dried in an electric oven.

Procedure

200 mg of β -CDP and 2.5 mL of buffer solution (pH 2.0) were added to a 100 ml stoppered conical flask at room temperature. The mixture was allowed to stand for 15 min. so that β -CDP should swell sufficiently and an appropriate amount of dye was added and made up to 75 ml with double distilled water. After the mixture was shaken in the thermostatic shaking water bath for 120 min. at the rate of 120 r.p.m., 5.0 ml of supernatant solution was transferred into a test tube and the absorbance was measured spectrophotometrically.

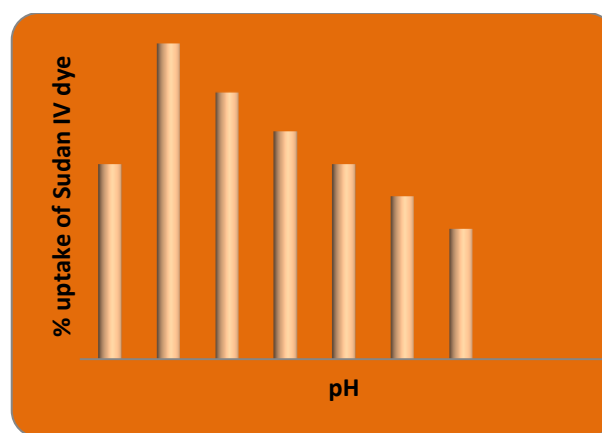


Figure 14. Effect of pH on the % uptake of the Sudan IV dye by the β -CD polymer

Effect of shaking time

The Shaking time is an important factor in determining the possibility of application of the β -CDP polymer for the selective % uptake of the Sudan IV dye. Different shaking time (ranging from 15 to 135 min.) were studied for the % uptake of Sudan IV dye by β -CDP polymer. The results of the % uptake of Sudan IV dye vs. the shaking time (Fig.16) show maximum % uptake ($\geq 95\%$) within 120 min. Therefore, the shaking time of 120 min. was selected as the adsorption equilibrium time.

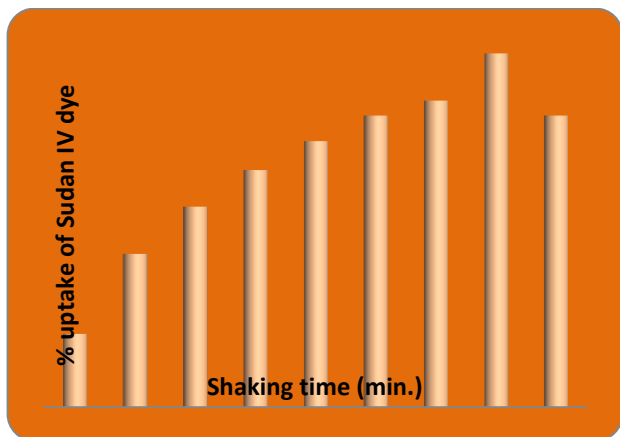


Figure 15. Effect of shaking time on the % uptake of the Sudan IV dye by the β -CD polymer

Effect of sample volume

In order to explore the possibility of enriching low concentration of analytes from large volume of solution, the effect of sample volume was studied on the retention of Sudan IV dye. For this purpose, 15, 30, 45, 60, 75, 90, 105 mL of sample solutions were taken. Quantitative uptake ($\geq 95\%$) was obtained for the sample volume of 75 mL (Fig.17). Therefore, 75mL of the sample solution was adopted for the preconcentration of analyte from sample solutions.

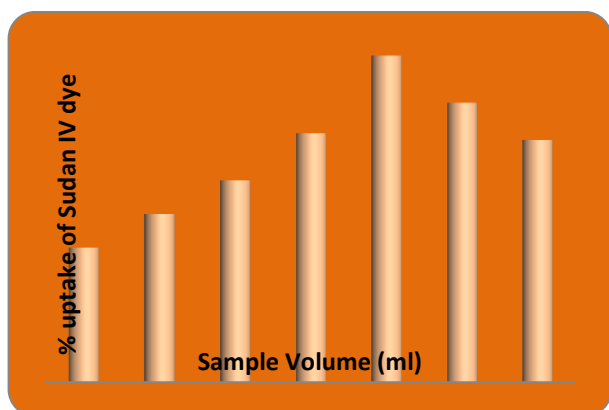


Figure 16. Effect of sample volume on the % uptake of the Sudan IV dye by the β -CD polymer

Effect of agitation speed

Speed of shaking is an important factor in determining the possibility of application of polymer for the quantitative % uptake of Sudan IV dye. Different speed (ranging from 40 to 140 r.p.m) were studied for the % uptake of Sudan IV dye by polymer. The results of % uptake of Sudan IV dye vs. agitation speed (Fig.18) shows that the % uptake reach maximum

($\geq 95\%$) at 120 r.p.m. Therefore, the shaking speed of 120 r.p.m. was selected for further studies

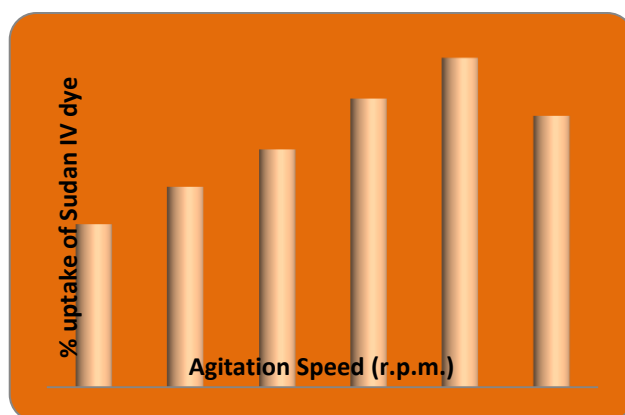


Figure 17. Effect of agitation speed on the % uptake of the Sudan IV dye by the β -CD polymer.

Effect of amount of polymer

The amount of the β -CD polymer is another important parameter that affects % uptake of dye. A quantitative removal ($\geq 95\%$) cannot be achieved when the β -CD polymer is less than the optimum amount. In order to optimize the smallest amount of polymer, 100 mg, 200 mg, 300 mg, 400 mg and 500 mg of the polymer were added to the solution containing known amount of dye. Quantitative recovery was obtained at 200 mg of β -CDP (Fig.19). Therefore, 200 mg of the β -CDP has been used for further studies.

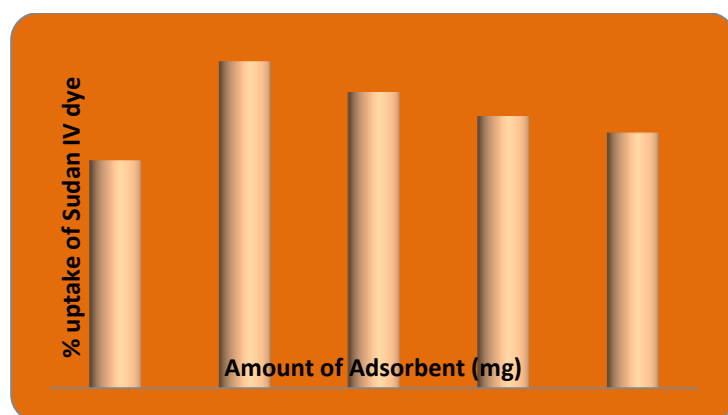


Figure 18. Effect of amount of adsorbent on the % uptake of the Sudan IV dye by the β -CD polymer

Applications

Determination of samples

The proposed method has been applied for the determination of Sudan IV Hot Chilli Tomato Sauce,

Hot Chilli Pepper and Red Sausage samples. The results are given in table.

Table 1. Result of determination of Sudan IV in food samples

Food Samples	Added, $\mu\text{g/ml}$	Found, $\mu\text{g/ml}$	Recovery, %
^a Hot Chilli Tomato Sauce	0	0.014	-
	0.324	0.325	96.15
	0.540	0.540	97.47
	0.949	0.950	98.65
^b Hot Chilli Pepper	0	0.010	-
	0.344	0.345	97.45
	0.560	0.558	97.89
	0.940	0.937	98.63
^c Red Sausage	0	0.011	-
	0.332	0.330	96.20
	0.546	0.545	97.84
	0.945	0.950	99.37

^aHot Chilli Tamoto Sauce, ^bHot Chilli Pepper and ^cRed Sausage - locally available in market.

IV. Conclusion

The proposed preconcentration method consist of a simple and low procedure which permits the quantitative recovery of brilliant green dye from food samples. The synthesis of the polymer is easy and the method has a good accuracy, sensitivity and repeatability. The polymer has been used in all the experiments performed for the study. It has a unique stability and reusability. This method is convenient for the determination of Sudan IV dye.

V. REFERENCES

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