

Partition Studies on Aqueous Two-Phase System using Sodium Dodecyl Sulphate and Ammonium Salts for Extraction of Dyes

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Summary: An aqueous two-phase system consisting of sodium dodecyl sulphate (SDS), an anionic surfactant, and NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ as salting out agent has been used for the extraction of ionic dyes. Transparent aqueous two-phase system is obtained when solution containing surfactant concentration (3 mL - 0.2 M SDS), NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ salt (0.8 g) and 1 mL of dye sample solution are mixed. The two-phase system thus obtained is of 3.0 ± 0.2 mL of upper phase and 1.6 ± 0.2 mL of lower phase. The partition behavior of dyes has been studied with various salts and results showed that among the studied systems, NH_4NO_3 - SDS and $(\text{NH}_4)_2\text{SO}_4$ - SDS systems were found suitable for dye extraction.

The effect of neutral, acidic and basic media on the extraction process has been investigated. Extraction of dye contents has been followed by UV-Visible spectrophotometric technique. Results showed that dyes (both anionic and cationic) were efficiently extracted into the upper phase.

Introduction

Aqueous two-phase system was developed in Sweden for the separation of macromolecules and different organelles [1]. These days different techniques using aqueous two-phase system have been developed for various materials [2]. Aqueous two-phase system involving solvent extraction [3], a conventional liquid-liquid extraction, has been effectively used for selective separation and pre-concentration of certain substances from others that exist in some matrices using an organic solvent for a liquid or solid sample. Extraction technique is widely used in the purification of industrial samples and removal of trace materials including heavy metals in the environmental and industrial matrices. The extraction technique sometimes requires large samples (by weight/weight, weight/volume and volume/ volume) and large volumes of extracting solvents such as benzene, chloroform, tetrachloromethane or other organic solvents. Use of such solvents is not appreciated now days because they are responsible for causing environmental pollution and health hazards.

Separation and purification of biomaterials such as enzymes and nucleic acids using aqueous two-phase extraction is very important in biological studies because of its merits and being beneficial over liquid-liquid extraction using organic solvents

[3]. In aqueous two-phase extraction technique, use of large volume of organic solvents is avoided to provide a mild extraction medium, which is more like a physiological environment and prevents reactive biomaterials from changing nature of essential quality. Such systems can also be applied for pre-concentration of solutes from small samples. The partitioning depends upon surface properties of molecules. The most important feature of aqueous two-phase system is that water content is high which provides suitable environment for biological materials [4].

A number of aqueous polymer two-phase extraction systems have been reported for the separation and purification of biomaterials [5-10] and metal chelates [11]. Tobacco protein separation has also been carried out by this technique [12]. Aqueous two-phase systems of cationic and anionic mixtures are also used for the separation of organic dyes [3] and porphyrine compounds [13]. Sodium dodecyl sulphate (SDS) is the most commonly used anionic surfactant. Among cationic surfactants, dodecyltriethyl ammonium bromide [3] and tetrabutylammonium bromide [14] are used. These surfactants as a single or as a mixture are used to produce aqueous two-phase system with and without salts acting as salting out agent in a given system.

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In the present study water based two-phase system consisting of SDS and NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ is proposed for the extraction of dyes. The dye sample is extracted into the upper phase completely.

Results and Discussion

Although several aqueous two-phase extraction techniques with surfactants have been proposed, the SDS – Ammonium salt system has not been previously investigated for the separation process. The two-phase formation conditions were examined and applied to the selective separation of dyes single as well as in the combined form.

The aqueous two-phase system is formed due to salting out effect of NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ on SDS when these are present in certain ratio i.e. 0.8 g of NH_4NO_3 or $(\text{NH}_4)_2\text{SO}_4$, 3.0 mL of 0.2 M SDS and 1.0 mL of dye sample (1.0×10^{-4} M) solution. After gentle mixing of contents the system was placed in water bath at 35°C until the two distinct phases are obtained. This phase development required 4-5 hours for NH_4NO_3 system and 7-8 hours for $(\text{NH}_4)_2\text{SO}_4$ system.

Effect of SDS and Salt Concentration on Volume of Two Phases in Aqueous Two-Phase System

In this study NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ were used as salting out agents. It was observed that these

salts behaved better than other studied salts such as NaCl, NaNO_3 and NH_4Cl for the phase separation. Figures 1 and 2 show change in volume of upper phase of aqueous two-phase systems that were obtained for SDS solutions (concentration range 0.04 – 0.5 M) on adding 0.3 g to 1.5 g of ammonium salts.

It is apparent from the results that aqueous two-phase systems are successfully achieved for higher SDS concentration and with enough NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ to produce excessive salting out effect. The volume of upper phase decreases and that of lower phase increases as the amount of salt is increased from 0.3 g to 1.5 g.

Partitioning of the two phases is a complex phenomenon which involves interaction between partitioned substances and the component of each phase. Various interactions involved are van der Waals forces, hydrophobic interaction, charge interaction and steric effects (which arise due to structure or geometry of the molecules) [15]. In the aqueous two-phase system under discussion, the SDS mostly moved into the upper phase while water in the upper phase could easily be extracted into lower phase by increasing amount of ammonium salt. This is presumably a cause of decreasing volume of upper phase with increasing amount of ammonium salt. The chemical species in upper and lower phases, which are formed when 3 mL of 0.2 M SDS, 0.8 g of ammonium salt and 1 mL of dye sample solution are

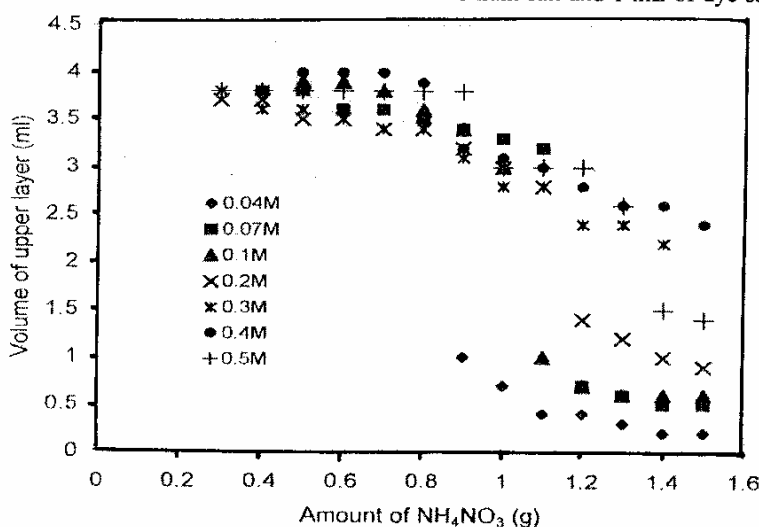


Fig. 1: Change in volume of upper phase in aqueous two-phase system by the added amount of NH_4NO_3 for 3.0 ml of each specific concentration of SDS (0.04- 0.50 M) and 1.0 ml of water as blank sample solution.

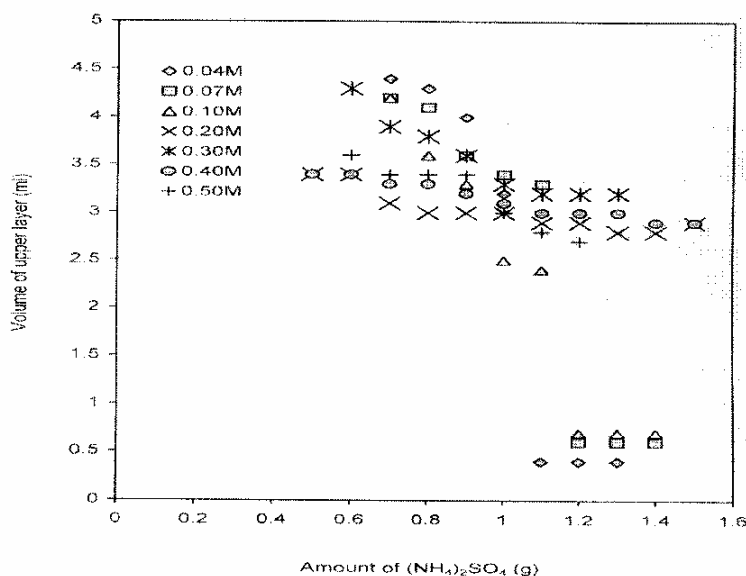


Fig. 2: Change in volume of upper phase in aqueous two-phase system by the added amount of $(\text{NH}_4)_2\text{SO}_4$ for 3.0 ml of each specific concentration of SDS (0.04-0.50 M) and 1.0 ml of water as sample solution.

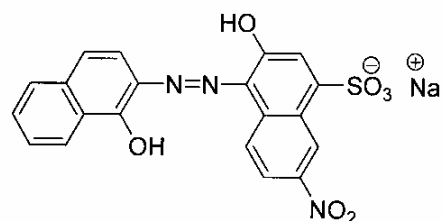
mixed, were identified using test tube chemistry. The results showed that NH_4^+ is present in greater amount in the upper phase as compared to the lower one while the reverse is true for Na^+ . This indicates that sodium cation associated with SDS salt remains in the lower phase while dodecyl sulphate anion goes in the upper phase. The chemical tests for NO_3^- and SO_4^{2-} (in separate systems) indicated their presence in greater amount in upper phase than that in lower phase.

The formation of described aqueous two-phase system can be obtained above 30°C , therefore, all the studies were made using water bath at 35°C .

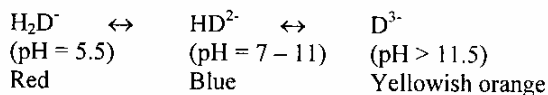
To apply this extraction system, following organic dyes were used for the extraction.

- 1- Eriochrome black T
- 2- Methyl orange
- 3- Methyl red
- 4- Thymol blue
- 5- Xylenol orange
- 6- Rhodamine B

Eriochrome black T (EBT) was chosen as model dye sample ($1.0 \times 10^{-4}\text{M}$) to be studied. The structural formula as sodium salt is,



Only dissociation of the two hydrogen atoms of the phenolic groups needs to be considered, so the dyestuff may be represented by the formula H_2D^- . The two pK_a values for these hydrogen atoms are 6.3 and 11.5 respectively. Below pH 5.5, the solution of EBT is red (due to H_2D^-), between pH 7 and 11 is blue (due to HD^{2-}) and above pH = 11.5 is yellowish orange (due to D_3^-) [16]:



The EBT was dissolved in aqueous two-phase system and then extraction procedure was performed. It was observed that EBT completely moved into the upper phase. The extraction of the EBT solution was studied at neutral, acidic and basic pH. For the study of UV-Vis spectra of EBT solution

(1.0×10^{-4} M) a known volume of upper phase (2.50 ml) and lower phase (1.5ml) were separated with the help of pipette. Both of these were diluted with de-ionized water up to 5.0 mL and 4.0 mL respectively. Absorption spectra were recorded in range 350- 750 nm using a matched pair of glass cells and water as a reference. The absorption spectra of upper and lower phases are given in Figures 3 and 4. A comparison of absorption spectra of upper and lower phases of sodium salt of EBT indicates that the dye is extracted completely into upper phase and is in the form of D^{3-} along with small proportions of HD^{2-} and H^2D^- . Absorption spectra of lower phase of EBT system show negative absorption in the range 350-630 nm.

The other selected dyes, Methyl orange, Methyl red, Thymol blue, Xylenol orange and Rhodamine B were also studied individually by following the same extraction procedure as discussed above for EBT (data is given in Table-1). The absorption spectra of lower and upper phases for these dyes were also recorded by following the procedure as given for EBT. A comparison of absorption spectra of upper and lower phases for each selected dye indicates the complete extraction into upper phase.

Density of Aqueous Two-Phase System

Densities of upper and lower phases of aqueous two phase system were measured, when 0.8 g of ammonium salt, 3.0 mL of 0.2 M SDS and 1 mL of dye were taken (at 35°C) and are given in Table-2. It is observed that density of upper and lower phases in water (as blank sample) system is greater than that of dye (as sample) system. Furthermore lower phase is denser than the upper phase.

Table-1: Volume of upper and lower phases after extraction in aqueous two phase system (For each extraction (at 35°C), 3.0 mL of 0.2M SDS, 0.8 g of ammonium salt and 1.0 mL of (1.0×10^{-4} M) of dye sample solution were used).

Dye	Salt	Upper phase (ml)	Lower phase (ml)
Eriochrome black T	NH_4NO_3	3.0	1.6
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6
Methyl orange	NH_4NO_3	3.0	1.7
	$(\text{NH}_4)_2\text{SO}_4$	3.1	1.6
Methyl red	NH_4NO_3	3.1	1.5
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6
Thymol blue	NH_4NO_3	3.0	1.6
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6
Xylenol orange	NH_4NO_3	3.0	1.6
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6
Rhodamine B	NH_4NO_3	3.1	1.5
	$(\text{NH}_4)_2\text{SO}_4$	3.0	1.6

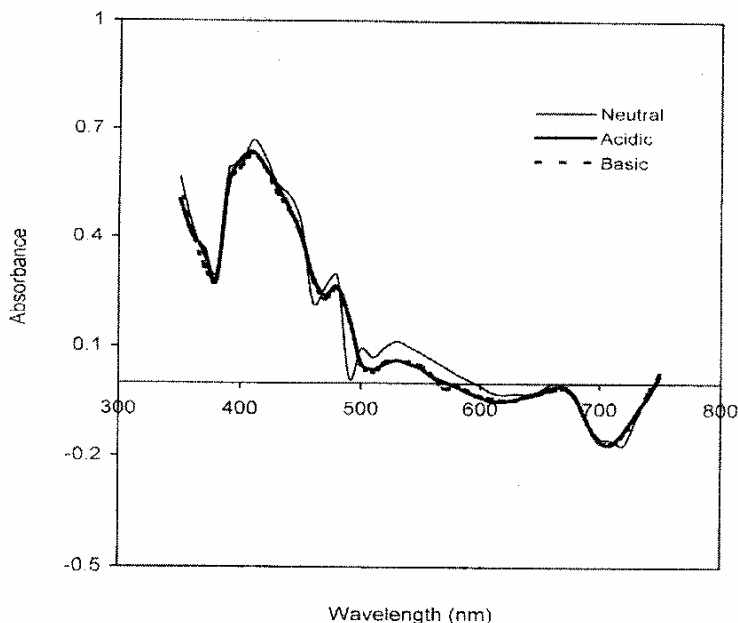


Fig. 3(a): Absorption spectra of upper phase of EBT at different pH in SDS- $(\text{NH}_4)_2\text{SO}_4$ system, pH of neutral solution = 7.0, acidic solution = 5.0 and basic solution = 12.0

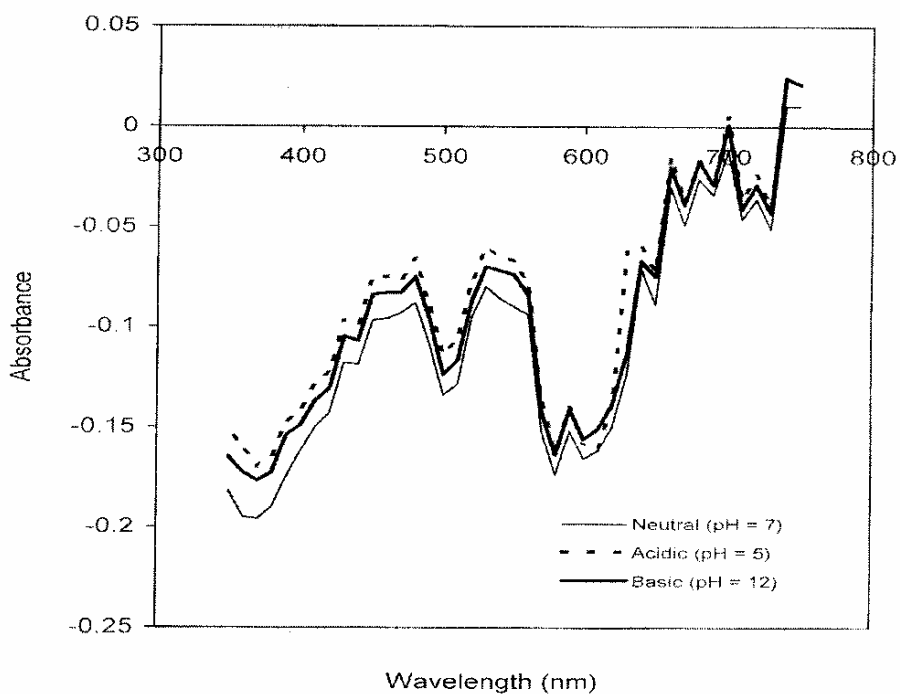


Fig. 3 (b): Absorption spectra of lower phase of EBT at different pH in SDS- (NH₄)₂SO₄ system. pH of neutral solution = 7.0, acidic solution = 5.0 and basic solution = 12.0

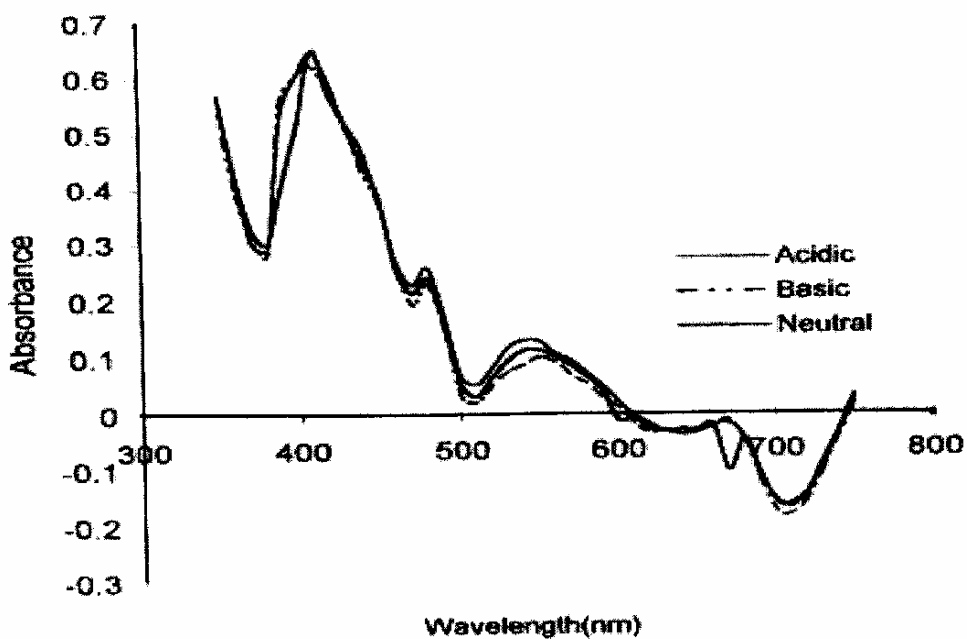


Fig. 4 (a): Absorption spectra of upper phase of EBT at different pH in SDS- NH₄NO₃ system. pH of neutral solution = 7.0, acidic solution = 2.0 and basic solution = 12.0

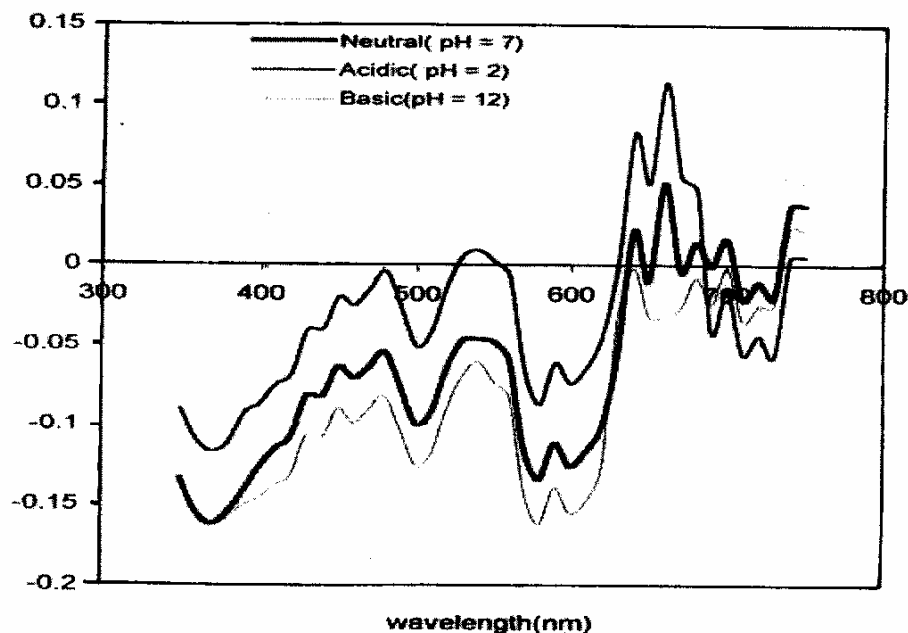


Fig. 4 (b): Absorption spectra of lower phase of EBT at different pH in SDS- NH_4NO_3 system. pH of neutral solution = 7.0, acidic solution = 2.0 and basic solution = 12.0

Table-2: Density data for aqueous two-phase system (For each extraction (at 35 °C), 3.0 mL of 0.2M SDS, 0.8 g of ammonium salt and 1.0 mL of (1.0×10^{-4}) M of dye sample solution were used.)

Sample	Phases	Density (g/ml)	
		NH_4NO_3	$(\text{NH}_4)_2\text{SO}_4$
Water (Blank sample)	Upper	1.007	1.070
	Lower	1.008	1.110
Eriochrome black T	Upper	1.002	1.060
	Lower	1.081	1.100
Methyl orange	Upper	1.003	1.060
	Lower	1.080	1.100
Methyl red	Upper	1.005	1.055
	Lower	1.075	1.090
Thymol blue	Upper	1.004	1.040
	Lower	1.080	1.085
Xylenol orange	Upper	1.004	1.050
	Lower	1.070	1.090
Rhodamine B	Upper	1.003	1.040
	Lower	1.070	1.100

Effect of Sample Volume and Amount of Ammonium Salts on Aqueous Two-Phase System

The effect of sample volume on extraction of dyes was studied with various amounts of ammonium salt used. The results are presented in Tables-3 and 4.

It is observed that increasing the sample volume up to 6.0 mL for 3 mL of 0.2 M SDS and 0.8

g of salt had no effect on the extraction and volume of upper phase increased accordingly with increase in the volume of the sample. However with sample volume 7.0 mL and onward, extraction is not complete and no distinct phase separation occurred. Volume of upper layer increased respectively with an increase in EBT volume (taken as sample) and that of lower layer remained approximately constant. It is concluded from these results that 6.0 mL (1.0×10^{-4} M) of EBT can be completely extracted into upper phase taking 3.0 mL of 0.2 M SDS and 0.8 g of ammonium salt as salting out agent. Therefore, it is necessary to increase the volume of SDS solution and also the amount of NH_4NO_3 / $(\text{NH}_4)_2\text{SO}_4$ for the large sample solution to obtain effective extraction. The other dyes also gave the similar results when extracted using this aqueous two-phase system.

The factors that govern the extraction of a substance in the two-phase system are various and complex. The important factors that influence the partition in aqueous two-phase system are hydrophobic property, bulk and charge of a molecule being partitioned. The results of present study suggest that anionic and cationic dyes could effectively be extracted from the aqueous solution

Table-3: The effect of sample volume on the extraction of EBT as sample using aqueous two-phase system. (For each extraction (at 35 °C) 3.0 mL of 0.2 M SDS was taken against the volume of dye and amount of NH_4NO_3 selected).

Amount of NH_4NO_3 Added (g)	Volume of EBT (ml)	Total Volume (ml)	Volume of Lower phase (ml)	Volume of Upper phase (ml)	Result/Remarks
0.8	0	3.6	1.8	1.8	All dye contents moved into upper phase
	1.0	4.6	1.6	3.0	
	2.0	5.6	1.6	4.0	
	3.0	6.6	1.6	5.0	
	4.0	7.6	1.6	6.0	
	5.0	8.4	0.4	8.0	
	6.0	9.4	1.2	8.2	
2.5	7.0	10.4	-	-	No distinct phase separation is observed.
	3.0	7.4	6.2	1.4	All dye contents moved into upper phase
	5.0	9.0	6.6	3.0	
7.0	11.4	7.0	4.6		
4.0	5.0	11.0	8.5	2.5	All dye contents moved into upper phase
	10.0	16.0	10.5	5.5	
	15.0	20.5	8.0	12.5	
7.0	15.0	23.0	10.5	12.5	No phase separation is observed

Table-4: The effect of sample volume on the extraction of EBT as sample using aqueous two-phase system. (For each extraction (at 35 °C) 3.0 mL of 0.2 M SDS was taken against the volume of dye and amount of $(\text{NH}_4)_2\text{SO}_4$ selected).

Amount of $(\text{NH}_4)_2\text{SO}_4$ Added (g)	Volume of EBT (ml)	Total Volume (ml)	Volume of Lower phase (ml)	Volume of Upper phase (ml)	Result/Remarks
0.8	0.0	3.6	1.8	1.9	All dye contents moved into upper phase
	1.0	4.6	1.7	3.0	
	2.0	5.6	1.8	4.0	
	3.0	6.6	1.8	5.0	
	4.0	7.6	1.8	6.0	
	5.0	8.4	1.6	7.0	
	6.0	9.4	1.6	8.0	
2.5	7.0	10.4	-	-	No distinct phase separation.
	3	7.4	3.2	4.2	All dye contents moved into upper phase
	5	9.0	3.4	6.0	
7	11.4	3.5	7.9		
4.0	5	11.0	6.0	5.0	All dye contents moved into upper phase
	10	15.0	5.1	9.9	
	15	20.0	5.5	14.5	
7.0	15	21.5	9.5	12.0	No phase separation.

with the proposed aqueous two-phase extraction system. In SDS- NH_4NO_3 and SDS- $(\text{NH}_4)_2\text{SO}_4$ two-phase extraction systems, the main factors that influence the extraction of a substance are considered to be the charge and the hydrophobicity of a substance being extracted.

Experimental

Reagents and Apparatus

All the solutions were prepared in de-ionized water ($\text{pH} = 7.0 \pm 0.2$), pyrex glassware was used in this work. Concentration of a substance partitioned in the aqueous two-phase system was determined using a Perkin-Elmer Lambda 3 B, UV-Visible spectrophotometer. The reagents used were purchased from different companies and used without further purification. Aqueous solutions of all the

reagents were prepared in de-ionized water (except methyl red and thymol blue) by taking the respective weights of reagents to a concentration of 1.0×10^{-4} M. For methyl red and thymol blue, the weighed amounts were first made soluble in minimum volume of methanol (4 mL) and then volume was made up to mark in 100 mL measuring flask with deionized water.

Procedure

In 10 mL measuring cylinders 0.8 g of NH_4NO_3 or $(\text{NH}_4)_2\text{SO}_4$ was taken. 3.0 mL of 0.2 M SDS and 1.0 mL of dye sample (1.0×10^{-4} M) solution was added to it. After gentle mixing of contents for about 30 seconds, the cylinders were placed in water bath at 35 °C until two clear phases were obtained. The phase development was completed in 4-5 hours for NH_4NO_3 system and 7-8

hours for $(\text{NH}_4)_2\text{SO}_4$. The two-phase system thus obtained has 3.0 ± 0.2 mL of upper phase and 1.6 ± 0.2 mL of lower phase. The dye sample solution is extracted completely into the upper phase as is being observed from the colour of two phases and also from spectrophotometric studies. An adequate volume (2.5 ± 0.2 mL) of upper phase and (1.5 ± 0.2 mL) of lower phase were pipetted out in separate 10 mL measuring cylinders and diluted with de-ionized water up to 5.0 and 4.0 mL, respectively. The extracted dyes were studied spectrophotometrically.

Conclusion

The SDS- NH_4NO_3 and SDS- $(\text{NH}_4)_2\text{SO}_4$ aqueous two-phase systems can be recommended for the extraction of organic dyes for large volumes of samples. Compared to some other extractive methods, the method described here offers following distinct features.

- a) - Process times (5-8 hours)
- b) - High extraction efficiency.
- c) - The chemicals used are not hazardous.
- d) - No use of organic solvents
- e) - The method is simple.

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