EXTRACTION OF ERICHROME BLACK-T DYE BY LIQUID-LIQUID-MICRO-EXTRACTION USING DEEP EUTECTIC MIXTURES

M. Tech. dissertation

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by

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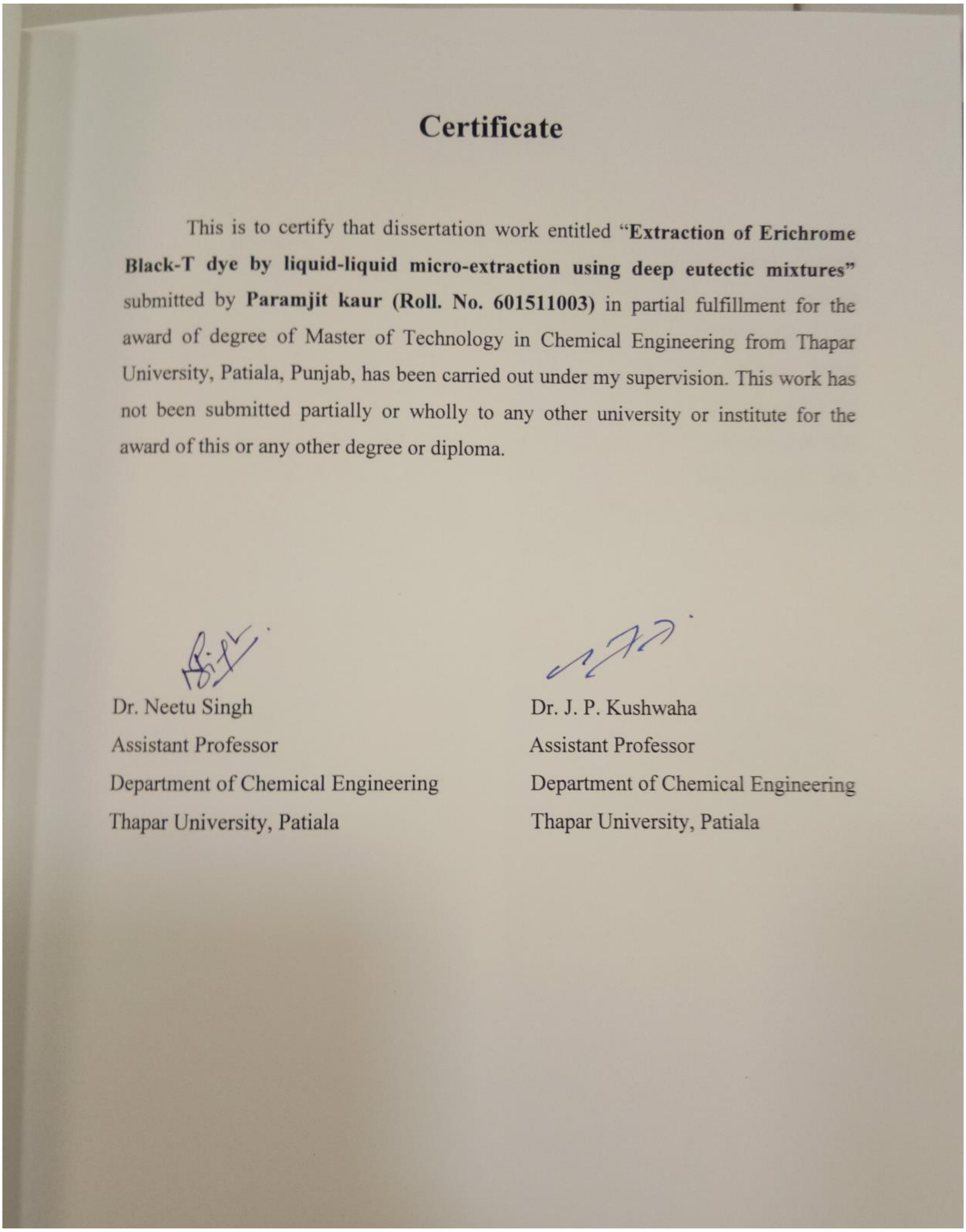

Certificate

This is to certify that dissertation work entitled "**Extraction of Erichrome Black-T dye by liquid-liquid micro-extraction using deep eutectic mixtures**" submitted by **Paramjit kaur (Roll. No. 601511003)** in partial fulfillment for the award of degree of Master of Technology in Chemical Engineering from Thapar University, Patiala, Punjab, has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of this or any other degree or diploma.

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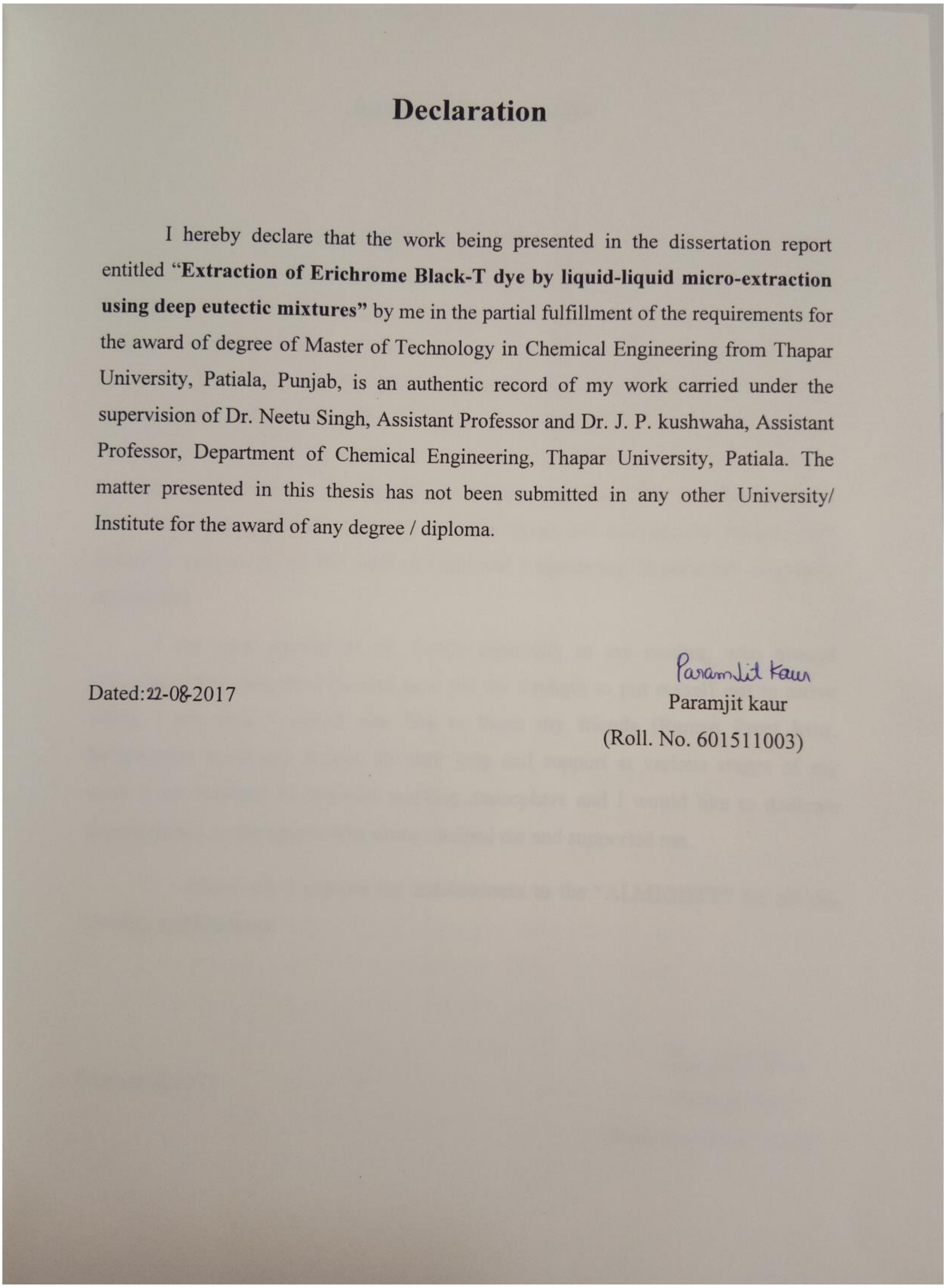

Declaration

I hereby declare that the work being presented in the dissertation report entitled "**Extraction of Erichrome Black-T dye by liquid-liquid micro-extraction using deep eutectic mixtures**" by me in the partial fulfillment of the requirements for the award of degree of Master of Technology in Chemical Engineering from Thapar University, Patiala, Punjab, is an authentic record of my work carried under the supervision of Dr. Neetu Singh, Assistant Professor and Dr. J. P. kushwaha, Assistant Professor, Department of Chemical Engineering, Thapar University, Patiala. The matter presented in this thesis has not been submitted in any other University/ Institute for the award of any degree / diploma.

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Abstract

Water is one of the greatest necessary natural source, unluckily misused the most by humanoid. The reasons behind this are, human population, increased living standards, rapid industrialization, and urbanization. Due to the color effluent (dye) large amount of wastewater is generated, now numerous forms of dyes are existing that are used in many productions for coloring the products most commonly synthetic dyes. These dyes are non-biodegradable and affect the human and aquatic life. For treatment of this wastewater various methods have been applied but they are also associated with many drawbacks.

Ionic liquids (ILs) have been used as novel extracting agent for dye removal from textile waste water. Although their clear benefits, maximum ILs are overpriced to prepare than organic solvents and are also non-bio degradable. Deep eutectic solvents (DESs), which is known as green solvent, presents physicochemical properties which resembles those of ILs. Other than this, they can be produced from low-priced, non-toxic, completely eco-friendly materials and their preparation is very humble.

Present study investigates the use of deep eutectic solvents (DESs) for the first time as extraction media for dye removal. Anionic dye Eriochrome black-T (EBT) was selected for this study by using Liquid-Liquid Micro-extraction technique. Glycolic acid & choline chloride (G:C), based DESs prepared in different molar ratios were used in this work. Extraction efficiency achieved in this study was quite comparable to ionic liquids for the same dye under similar operating conditions. While comparing the performance of different DESs, G:C (1:1,2:1,3:1) achieved the higher efficiency. Effect of different relevant parameters (solution pH, volume of DES and initial dye concentration) on extraction efficiency was also studied. From this study it can be concluded that, DESs can be used for extraction of dyes from textile effluent. There is need to explore other DESs also other than used in this study for this purpose. The prepared DES was characterized by FTIR and NMR spectroscopy.

Key words: Liquid-Liquid micro-extraction; Deep eutectic solvents; Erichrome Black-T dye

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Nomenclature

AAS	Atomic absorption spectrometry
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CPCB	Central Pollution Control Board
CSE	Centre for Science and Environment
DLLME	Dispersive liquid–liquid micro-extraction
EF	Extraction efficiency
FID	Flame ionization detection
GC	Gas chromatography
GFAAS	Graphite furnace atomic absorption spectrometry
HPLC	High Performance Liquid Chromatograph
IL	Ionic liquid
LLE	Liquid-liquid extraction
LLME	Liquid–liquid micro-extraction
PAH	Polycyclic aromatic hydrocarbons
RSD	Relative standard deviation
SS	Suspended solids
SPME	Solid-phase micro-extraction
DEM	Deep eutectic mixture
HBD	Hydrogen bond donor
HBA	Hydrogen bond acceptor
EBT	Erochrome black-T
GI	Glycerol
FTIR	Fourier transform infrared Spectroscopy
NMR	Nuclear magnetic Resonance

CHAPTER 1 INTRODUCTION

1.1 General introduction

Water is one of the most essential natural resource. About 70% of earth being covered with water unfortunately exploited the most by humans. The most common reasons behind this are human population, rapid industrialization, urbanization and increased living standards. Today with the rapid expansion of cities, most of water is used in domestic purposes and remaining consumed in agricultural and industrial activities. Due to the unrestricted and excessive exploitation of water, today the whole world is facing water crisis. The shortage of fresh water increased due to the industrial activities play the lead role to produce the problems of environment pollution via industrial waste water discharged into the receiving aquatic bodies. Although the treatments of these industrial wastewaters before discharge is obligatory by legislations of relevant environment protection, approximately in world industrial facilities don’t have sufficient treatment plants for wastewater. As a result the receiving water quality is deteriorating rapidly. Shortage of water makes pressure on the manufacturers to wage extra for consumption of fresh water and production of wastewater. Above all manufacturers are forced to consider the reuse and recovery of wastewater.

Nowadays, the contamination due to color, dyestuff and industrial wastewater is becoming the main problem for our environment. Dyes are extensively used in different industries for coloring their products in different process such as plastic, paper, food, pharmaceuticals, leather, textile etc. by increasing the industrialization, consumption of fresh water and the generation of wastewater was increased , it affects the environment and human health directly or indirectly by discharging the wastewater into water sources. Textile industries are the most polluting industrial plants, it contains different types of dyes out of which some are highly toxic, hazardous and mutagenic and also minimize the photosynthesis activity & light penetration [1].

1.2 Dyes

Dyes are extensively used in coloring businesses such as fabric, food, cosmetics, paper, letterpress, fleece and plastics, fabrics producers are the main user of dyes. Mostly industrial sector, such as plastics and paper, dyestuffs, textile use dyes to shade their products and similarly consume a large quantity of the water. At the end, they produced a massive quantity of colored wastewater into the environment. It is researched and reported that color in water is the initial and first pollutant to be predictable in the wastewater easily [2]. In the whole world textile industries are biggest consumer of synthetic dyes, approximately 10,000 different types of synthetic dyes are available and 7×10^5 tons consumed by textile industries per year in the entire world [3].Dye wastewater is mixture of many pollutants and chemicals which have complex molecular structure, it increase the organic load and interrupt the ecological balance of the receiving water source. Approximately 93% colored wastewater comes out from textile industries including heavy metals and high concentration of organic compounds [4].

The major structure element of dye are chromophore and auxochrome.In which chromophore group is a color provider represented by azo (–N=N–); carbon (=C=C=); carbonyl (=C=O); nitro (–NO2 or =NO–OH) groups and auxochrome increase the color intensity of the chromogen. Amine, carboxyl, sulfonic radicals and hydroxyl are present in this group.

1.3 Classification of Dye

Dyes are mainly classified as natural and synthetic dyes.

1.3.1 Natural dye

Natural dyes are organic compounds used to color different types of products. In 1856, natural dyes or colorants were extracted from vegetables, plants, insects and minerals such as roots, barks and leaves. However due to increasing residents and manufacturing activities, natural colorants do not complete the manufacturers demand. Their characteristics have been inadequate mostly in food trade [2].

1.3.2 Synthetic Dyes

In 1856 William Henery Perkin revealed the first synthetic dye. These colorants are prepared from synthetic assets which include earth minerals and by-products of petroleum [5]. The dye effluents produce the serious environment and health hazards because of their composite molecular arrangement as well as synthetic origin. Synthetic dyestuff are several types like acid dyes, direct dyes, vat dyes, basic dyes, disperse dyes and reactive dyes [6].

1.3.2.1 Classification of synthetic dyes

Various types of synthetic dyes are available into the market, which can be mainly divided into three types: cationic, anionic and non-ionic dyes. Anionic dyes are categorized into azo dyes, direct dyes, acid dyes and reactive dyes while cationic dyes are the basic dyes. Classifications of dyes and classification with utilizations are illustrated in Table 1.1.

Acid dyes

Acid dye are very important and large group of dyestuff. Anionic dyes are soluble in water and directly applied to wool, silk, nylon fiber. The first acid dyes were combinations of basic dyes with nitric or sulphuric acid groups. The dyeing process is reversible and Most of the acid dyes contain anthraquinone or triarylmethane and azo type of chromophore groups. Acid dyes are not applicable to cellulosic characters. Several man-made food shades are involved in it.

Basic dyes

Basic dyes (cationic dye) are also solvable in water that are generally useful to acrylic fibers and mostly used with mordant. By mordant, basic colorants are used designed for yarn, linen, acetate, nylon, polyesters, acrylics and modacrylin, wool and silk are also used in the coloration of paper. Methyl Violet, Crystal Violet, Rhodamine, Magenta etc. are very common examples of basic dyes.

Direct dye

Direct colorants are water answerable anionic compound that are used directly to cellulose lacking of the use of mordents. They are applied for coloring cotton, wool, silk, nylon, rayon, paper and leather and are also used as a pH indicator. Direct dyes are used on cotton, paper, leather, wool, silk and nylon. Congo red is an example of direct dye.

Mordant dyes

The mordant or chrome colorants are bitter in character which require a mordant. The mordant Sodium or potassium bichromate is used with them in the color bath or after the course of coloring is finished. Mordant dye used for cotton, linen, silk, rayon and nylon but are least effective for them. Many heavy metal mordants are hazardous to health. Most mordant dyes are azo, oxazine or triarylmethane compounds.

Disperse Dyes

Disperse dyes are water-insoluble and established for the coloring the cellulose acetate. Disperse dye used for coloring the polyester and also used to dye nylon, cellulose triacetate and acrylic fibers. The dyes are finely crushed in the occurrence of a dispersing agent and sold as a paste, or spray-dried and sold as a fine particles.

Vat dyes

Vat dyes are water unsolvable dye and cannot be straight used to fibers. However, these are used for making soluble alkaline reduction solution and for affix the textile fiber. This dye is restored in insoluble form for decreasing the effect of oxidation or exposure to air. Indigo dye is the unique vat dye. These colors are greatest for linen, rayon and cotton fiber.

Reactive dye

This dye can be used directly to the fibers. Covalent bonds present in dye attaches reactive dye to natural dye make this dye permanent. Some reactive dyes are applied at room temperature and are easy to use such as Cibacron F, Procion MX, and Drimarene K. Mostly reactive dyes are used for cotton dyeing.

Sulfur dyes

Sulfur dyes are applied for coloring the cotton in dark color. In a solution of organic compound, fibre is heated for effective dyeing. Some derivatives are used like nitrophenol, and sulfide or polysulfide. The biological compound responds with the sulfide source to form dark dyes that adhere to the fabric. The most famous and marketing sulfur dye is Sulfur Black 1, of which defined chemical structure is not defined properly.

Table 1.1: Classification of dyes on basis of their utilizations

Name of dyes	Utilization
Acid dye	Wool, silk & Nylon
Basic dye	Acrylic, jute
Vat dye	Viscose, cotton
Disperse dye	Nylon, Acrylic, polyester, Triacetate, Di-acetate
Direct dye	cotton, Viscose
Reactive dye	Cotton, silk, wool, Nylon &Viscose
Sulfur dye	Cotton, viscose, wool & silk
Azoic dye	viscose, cotton
Mordant dye	Cotton, silk & wool

1.4 Dyes Used In the Fabric Manufacturing Process

Textile industries are the largest and oldest industry in India. In the world after china, India has the biggest industry in the country, 14% contributes in the production for country and 17% export [7]. The world's consumption of dyes is described in figure 1.2. In textile industries different types of dyes are used. The textile industries are divided into three parts viz., woolen, cotton and synthetic fibers which depends upon the raw material used. In textile dyeing and printing include pretreatments, printing/dyeing, finishing and some other methods. In pretreatments desizing, scouring, washing and other methods are involved and discussed following.

- **Sizing** is the first step of preparation, which involves the sizing agents for providing the strength and minimize the breakage of the fiber sizing agents are added such as polyvinyl alcohol, starch and carboxymethyl cellulose.
- **Desizing** the step is used for the removal of sizing materials that prior to weaving.
- **Scouring** are used for removing the impurities by applying alkali solution (mainly sodium hydroxide) to breakdown fats, natural oils, waxes and surfactants .
- **Bleaching** is used for removes the unwanted/extra color from the fiber by applying different chemicals, which are sodium hypo chloride and hydrogen peroxide.
- **Mercerizing** step is used to increase the dye luster and ability .This is a continuous process in which, before the dyeing process fiber washes with acid and a concentrated alkaline solution applied.
- **Dyeing** step is used for coloring the fiber, this process required large amount of water, not for the dye bath but for rinsing step. In this step various chemical such as salts, metals, organic processing aids, surfactants, formaldyde and sulphide are added for dye adsoption onto the fiber. In dying process approximately 10-25% textile dyes are lost during processing and pollutants mainly organic matter are also obtained from the pretreatments methods.

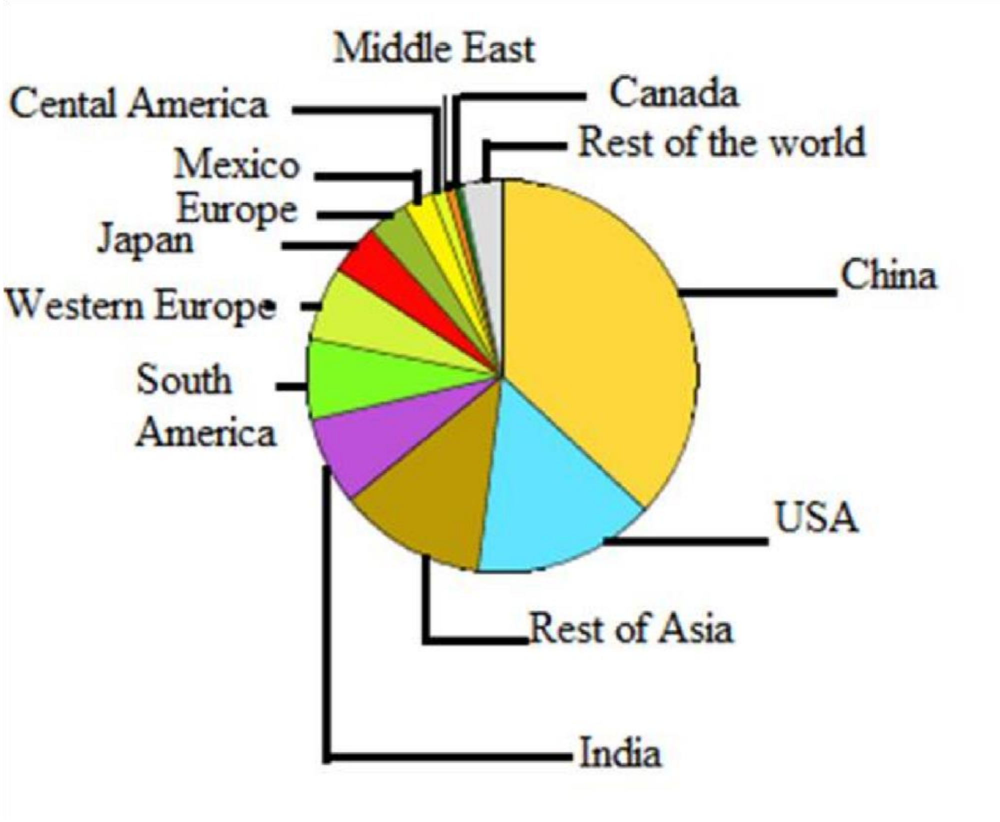


Figure 1.1: World consumption of dyes in 2007 [8]

In wet processing industries a large volume of water is consumed for dyeing and washing process. Reportedly in dyeing and chemical operation 30-40% water is used. Remaining 60-70% water is used in washing stage. It was estimated that in 1994, 502 textile industries discharged 40,000m^3/day discharge wastewater which produced the 26,000 kg/day BOD load Table 1.2. According to another source 8 m^3/day discharge in small scale individual dyers and corresponding the value for large scale dyers 70-400 m^3/day [8]. Approximately 1,28,700 m^3/day produce wastewater for 1430 tons/day products knit process.

According to the sources, the annual production of sewage in China is already reached at 390 million tons, which include 51% industrial waste, with the amount of 1% growing every year and near around 70 billion tons of dye wastewater produced by fabric and coloring industry [9].

Table: 1.2 The potential specific pollutants from textile dyeing and printing

Process	Process Compounds
Desizing	Sizes, starch, enzymes, ammonia, waxes.
Scouring	Disinfectants and insecticides residues, NaOH, soaps, surfactants, fats, oils, waxes, pectin, sizes, anti-static agents, enzymes and spent solvents.
Bleaching	AOX, H_2O_2, high pH, sodium silicate or organic stabilizer.
Mercerizing	NaOH, High pH.
Dyeing	Color, Metals, colors, salts, organic processing assistantssurfactants, sulphide, acidity/alkalinity and formaldehyde.
Printing	Solvents, Urea, metals and color.
Finishing	Resins, Waxes, resins, chlorinated compounds, stearate, acetate, softeners and spent solvents.

1.5 Wastewater remediation

Wastewater effluents are difficult to treat and hazardous to the environment because their composite molecular arrangement and synthetic source. A series of methods and technologies are settled for the removal of dyes. These methods are mainly distributed into three main groups Physical, Chemical and biological methods. Each method has its own advantages and drawbacks on basis of their design, cost and efficiency.

1.5.1 Physical process

There are number of Physical methods existing for dye removal from water and wastewater streams. Most commonly used methods are membrane filtration processes which contain Nano filtration, Reverse osmosis, Electro dialysis and Adsorption techniques.

Adsorption process

Adsorption is the most effective and economical method for removing the dyes into the wastewater. The process also used for remove the organic dye and other toxic chemicals such as cyanides, phenols and pesticides. The adsorption process is effective separation method and the advantages of this process such as low initial cost, ease of operation, simplicity of design and unresponsive to toxic materials [2, 11]. The effectiveness of adsorption is determined by the adsorbate, adsorbents and its physical and chemical possessions. The selectivity of adsorbents is based on few features such as total cost, obtainability, external area and adsorption capacity. The most commonly used adsorbents are activated carbon which is manufactured from carbonaceous material such as coal, wood, petroleum products etc.

Activated Carbon

For dye removal this method was commonly used and found effective for removing direct, vat, dispersed, pigments, acid and reactive dyes. The performance of process is depended on some characteristics such as, kind of carbon used and physical appearance of wastewater and massive dose of carbon. For improving the removal rate regeneration and massive dose of carbon is used. Several other dye removal methods like activated carbon are used for particular waste .Activated carbon method is very costly. In this method the carbon is reactivated and the recurrence results shows 10±15% loss of the sorbent.

Membrane Filtration

This method is used to separate and clarify the dye from effluent. This process has some important features such as an adverse chemical environment and resistance to temperature but has some drawbacks disposal problem of concentrated residue after the separation, high capital cost, membrane replacement and clogging. Some other methods those are economically and at lower cost are available such as, ultrafiltration, Microfiltration and Nano filtration are considered for chemical and biochemical processing [12]. Nano filtration membrane is used for removing Methylene blue and cotton dye effluent. It is a mixture of ultrafiltration and reverse methods [13, 14]. Nano porous membranes have been used for Victoria Blue, Methyl Violet and Rhoda mine [15].

Ion exchange

This method are not commonly used for the treatment of colored effluent, generally due to the estimation that ion exchangers cannot accommodate a wide-ranging of dyes. The main benefits of this methods are, there is no adsorbent loss on regeneration, and soluble dyes removed and recover the solvent after use. Organic solvents are expensive, so that the cost are main disadvantages of this process. For disperse dyes this methods are not effective. The ion exchange method are applicable in alcohol, sugar, pharmaceutical and waste water fields [16] has been reported. Ion exchange method is a virtuous for separate and process to distinct and remove toxic and soluble colors from waste water although the high capital cost related with this method limited its use.

1.5.2 Chemical methods

Chemical methods contains various methods such as coagulation or flocculation joined with filtration, precipitation- flocculation with Fe(II)/$Ca(OH)_2$, Electro kinetic coagulation, conventional oxidation techniques by oxidizing agents (ozone), irradiation, electro-flotation or electrochemical methods. For dye removal from dye waste water and effluent these methods are used. These methods are very expensive and produced disposal problem of concentrated sludge hence these methods are not much used. These techniques are very effective for the treatment of wastewater but due to certain causes like excess electrical energy demand, bulk amount of chemical mixtures required and high priced, these methods are not used.

Advanced Oxidation Processes

These methods contains the methods such as Fenton's reagent oxidation, sonolysis and ultra violet (UV) photolysis has ability to degrade the organic effluent at ambient pressure and temperature [17]. For the removal of textile dye effluent and organic component recalcitrant AOP used. AOP advantages over the other treatment methods are organic non-biodegradable component removed, no problem of sludge production [18]. For removal of dyes several advanced oxidation methods have been employed.

Ozonation

Ozonation method was used for the reactive dyes removal from the industrial textile effluents at temperature of batch reactor was 35°C. The effect of reaction time and pH was also considered on the efficiency of decolorization. The decolorization efficiency was increased as the time process increased, after the 6 hour greater than 90% color removed [19]. For color removal ozonation method was very viable. Ozonation was carried out in a semi-batch reactor for eight reactive dyes mixtures which is concentration of dyes lies between 50-500 mg/L. COD and color removal depends on the dose of ozone, maximum COD and color removed at the dose of 4.33 mg/l at 30 min of ozone. At low concentration of dye, COD and color removal was very fast .As increased the concentration from 50-500mg/l rate of decolorization decreased. Biodegradability increased in Ozonation by following the pseudo first order kinetics. Chloride, sulphates, nitrates by products formed as the result of cleavage and oxidation of substituent groups present in dye molecules [20].

Electro kinetic coagulation

Electro kinetic coagulation (EC) is used for treatment of the wastewater. In this method the aluminum and iron metal electrodes are immersed into water effluent with direct power source and metal plates are dissolution into the effluents [21]. EC method was simple, low cost and reliable method for dye removal such as reactive blue, disperse red and direct red [21,22]. This method has various advantages but the main advantages are there is no generation of secondary pollution as compared to other conventional techniques. The disadvantages obtained from this method are large amount of sludge produced and required further treatment by filtration and flocculation [23].

1.5.3 Biological treatments

The biological treatment methods are cost effective, less amount of sludge produces and environment friendly. Biological methods aerobic and anaerobic, mainly used for the waste removing from the wastewater effluents that enriched with organic molecules. Dyes molecules are difficult to biodegrade because they are stable organic molecules. Some other factors that effected the biodegradation of dye are involving high molecular weight, complex structure and highly water soluble which decreases the penetration by biological cell membrane [8]. Dye removal from industries and textiles by biological methods can be divided into three methods, aerobic treatment, anaerobic treatment, and combined anaerobic–aerobic treatment.

Anaerobic Degradation process

Anaerobic degradation process used for dye removal and performed in the nonexistence of oxygen. Anaerobic digestion course has ability to decompose the organic complexes so that they can be also used in aerobically or new treatment methods of dye exclusion [24]. The biodegradation progression considered at decolourization phase wherever the azo links double bonds of nitrogen are breaking by micro-organiums and at second stage aromatic amines degrade [25]. Under anaerobic conditions decolourization stage occurs. Researchers investigated under anaerobic conditions bacteria used for reduction of dyes mainly involving Methyl orange (MO) and Naphthol green B (NGB) by Shewanella oneidensis MR-1[26] and Reactive red by Halomonas variabilis and Halomonas glaciei [24]. The procedure has also some disadvantages like the process required the further treatment and poisonous by-products produced. Thus a mixture of anaerobic and aerobic method is suggested for the biodegradation of textile colorant.

Aerobic Degradation

Under aerobic conditions, Fungi and bacteria are those microorganisms used for dye removal. Bacteria has ability to culture and grow rapidly than fungi has able to metabolize chlorinated and new organic pollutants [24]. For complete decolourization and degradation fungi and bacteria are used for textile effluent have the several advantages over the other treatment methods [27]. However, this course is very slow and not appropriate for the actual waste of textile.

Table 1.3: Drawbacks of different dye wastewater treatment processes

Physical method	Chemical method	Biological method
High capital cost, incapability in treating large volumes of wastewater, Pressure fluctuations, lack of efficiency, chronic toxicity, production of sludge (which demands processing too), short membrane life due to clogging [5].	These process are very pricey and unattractive. Large requirement of electrical energy and chemical reagents consumption are common difficulties of these methods [1, 3].	Requirement of a specialized equipment for efficient dye removal as these processes are quite energy demanding and generate a considerable amount of byproducts [6]. These processes are unable to degrade recalcitrant azo dyes completely due to their xenobiotic complex chemical structures and synthetic organic origin [21, 5].

1.6 Problem statement

Researchers found various ways for dye removal from wastewater; all these available methods are associated with many drawbacks. Recently Ionic liquids (ILs) have been used as novel extracting agent for dye removal from waste water. Despite many advantages, ILs are associated with many limitations, they are very expensive to arrange than other organic solvents. Similarly, the ILs effect on environment is essentially reliant to the structure of anion and cation, significance that ILs are not completely green. Thus, there is a need to use novel solvents which are more suitable, economical and can offer excellent properties like, non-toxicity, biodegradability, good thermal stability, water compatibility, environmental friendliness and non-flammability. In view of the above requirements deep eutectic solvents (DESs) have been proposed to use as extracting agent, subsequently their physicochemical properties be similar to ILs. DESs can be formed from economical, non-hazardous, totally eco-friendly constituents and their production is very humble, necessitating only heating and mechanical stirring to accomplish the production in one step.

In this study it is proposed to explore the opportunity of consuming deep eutectic solvents (DESs) as extracting agent for abstraction of EBT dye using LLME technique. Glycolic acid and choline chloride based DESs in different molar ratios will be used for this purpose, and their performance will be compared.

1.7 Aim & Objective

The target of this research work is to investigate the performance of deep eutectic solvents for the removal of EBT dyes by considering several parameters.

Objectives: The main objectives in this study are as follows:

1. To prepare and characterize the glycolic acid & choline chloride based deep eutectic solvents (DESs) of different compositions.
2. To extract the indicator dyes; Erichrome black-T dye by using Liquid-Liquid micro-extraction technique.
3. To study the comparative performance of different DESs in terms of dye extraction efficiency by varying relevant process parameters like initial dye concentration, solvent dose and pH of solution.

2.1 Dyes and their toxicity effects

In dye containing industries, the textile industry is most heavy polluter of generating the wastewater, gas, noise and solids. Dye wastewater damaging the environment and natural resource, which consume large quantity of fresh water and generate huge volume of waste water. Thousands different types of dyes are available in textile industries but the azo dye used maximum. Approximately the annual production of dyes is one million metric ton , which 70% on weight basis azo dye represent .They are identify and characterized by azo bonds(-N=N-) which are associated with one and more aromatic systems. Azo dyes are most important class of dyes, currently about 2000 dissimilar types of azo colorants are used in many applications.

The concentrations of dye in wastewater is higher 1mg/L because of direct discharge the textile effluents into water resources without treated. In wastewater dyes are present in high concentration that stop sunlight and disturb the biological system of the aquatic bodies and also effects the photosynthesis process of algae and aquatic plants [28].

The color in water streams is recognized as an aesthetic problematic rather than an eco-toxic hazard. So that the humans accepts the green, brown and blue colors of water sources but not the non-natural colors as like purple, red .The hazardous and infecting properties of dyes on environment and marine bodies can caused by long time existence of dyes in atmosphere. Due to complex structure and synthetic nature of dyes, mainly aromatic, various dyes non-biodegradable nature and having carcinogenic action or causing dermatitis, allergies, skin irritations. Azo dyes mainly have the aromatic compounds, showing the toxicity effect of chronic and acute. Frustration or dissimilar tissular changes. Mostly azo dyes, have complex structure and aromatic compounds those represent the acute and chronic toxicity. Azo dye adsorption caused by several health problems such as gastrointestinal tract, lungs, skin and also formation of hemoglobin adducts and disturbance of blood formation [29].The carcinogenicity of azo dyes, which found a significant amount of textile dyes identified [30,31]. Some azo dyes are caused for the bladder cancer in human being and splenic sarcomas, hepatocarcinomas and nuclear anomalies in investigational animals and to reason chromosomal aberration in mammalian cells [32, 33]. It has been reported that various azo dyes caused to damage the genetic material that can responsible for the origin of malignant cancers. Some azo dyes such as Direct Black 38, azo dye.

Cationic dyes can also cause of several health problems which include vomiting, quadriplegia, cyanosis, jaundice and Heinz body formation [34].

Table: 2.1 Central pollution control board (CPCB) standards for Dye and dye intermediate industries

Parameters	**Standard** **(applicable for all modes of disposal)** Maximum concentration values in mg/l except for pH, color, Bioassay and Temperature		
Treated effluents	**Discard in water**	**Marine water**	**On land for irrigation**
pH	6.5 to 8.5	5.5-9.0	5.5-9.0
Suspended solids	100	-	200
Color (Hazen unit)	400	-	-
Bio-chemical oxygen demand [3 days at 27°C]	30	100	100
Chemical oxygen demand (COD)	250	250	-
Total chromium as Cr	2.0	2.0	-
Sulphate as (SO_4^{2+})	1000	-	-
Ammonical nitrogen as Nitrogen	50	50	-
Mercury (Hg)	0.01	0.01	-
Copper	2.0	3.0	-
Zinc(Zn)	5.0	15.0	-
Nickel(Ni)	3.0	5.0	-
Lead(Pb)	0.1	2.0	-
Mangese(Mn)	2.0	2.0	-
Chloride	1000	-	-
Phenolic compounds as C_6H_5OH	1.0	5.0	-
Oil & grease	10.0	10.0	-
Bio-assay test (with 1:8 dilution of effluents)	90% survival of test animals after 96 hours in 100 effluents	-	-
Temperature	Shall not exceed 5°C above the receiving water		-

Emission standards (process)	
Parameter	**Limiting concentration in milligrams / Normal cubic meter (mg/Nm^3) unless otherwise stated**
Oxides of sulphur (SO_x)	200
HCl (Acid mist)	35
Ammonia (NH_3)	30
Chlorine (Cl_2)	15

2.2 Liquid-liquid extraction (LLE)

Liquid-liquid extraction is a commonly used separation progression in which ability of a solvent is used to selectively separate one of the compound from the mixture. This technique is used where distillation failed, for example: temperature-sensitive components and azeotropic mixtures. In this separation process liquid solvent phase is added into the original liquid mixture so that it component of interest can be extracted. These two phases are chemically different, one phase dispersed into the other by droplets. Which is one phase is water and other is organic. The equipment used for extraction process are mixer settler and extraction columns [35].

2.2.1 Drawbacks of LLE

- Uses large amounts toxic organic solvents.
- Preparation methods are expensive.
- Time-consuming method.
- Environment unfriendly.
- Multi-stage operation.

2.3 Liquid–liquid micro-extraction (LLME)

Micro-extraction is a liquid-liquid extraction technique, in which volume of solvent is used in microliter. Solid and semi-solid materials are extracted and analyzed by solvent micro-extraction (SME) and solid-phase micro-extraction (SPME). Both methods has differences but they share same features because in both are micro-extraction process applied. Both methods has many advantages like, it’s very simple and easy to prepare, low cost, effectiveness, solvent used in minimal amount and excellent abilities to clean up samples [36].

In 2006, Rezaee et al. established dispersive liquid–liquid micro-extraction (DLLME) for pre concentration of polycyclic aromatic hydrocarbons (PAHs) in water sections. The first DLLME techniques employ a combination of a high-density abstraction solvent, a water-miscible solvent, and a polar disperser solvent [37]. The abstraction solvent must be capable to extract analyses, is solvable in the disperser solvent, unsolvable in aqueous samples, and have density greater than that of water. The disperser solvent should be soluble in equally water and removal solvent. LLME is simple, rapid, and inexpensive technique. It requires less volume of sample, and provides high extraction efficiency (EF). However, the high-density extraction solvents which include chlorobenzene, chloroform, or carbon tetrachloride and are highly toxic [38]. It can be applied in the analysis of organic compounds (pesticides, pharmaceuticals, and phenols) and inorganic analytes (Cu, Pb, and Cd). DLLME methods have been used in the analysis of various samples such water samples, food, urine, animal tissue or offal, soil, and leaves [39, 40] which were discussed following.

Gharehbaghi et al. [41] studied the dye removal by IL based dispersive liquid-liquid extraction method. They extracted Congo red (a carcinogenic textile dye) from aqueous solutions by using a binary solution, compassing the extraction solvent (ionic liquids) and a disperser solvent. The binary mixture was quickly inserted into CR dye containing water sample. Then cloudy solution was obtained, and maximum dye molecules were extracted and detached from aqueous phase. The effect of different parameters such as type, pH, amount of IL, initial dye concentration, volume of the dispersant, and concentrations of salt were also studied.

Sanagi et al. [42] studied the Analysis of antidepressant Drugs in water by Dispersive Liquid-liquid Micro-extraction Based on Solidification of Floating Organic Droplet. In this process organic solvent (low density and less toxicity) used by consuming disperser solvent (0.5 mL acetonitrile) having 30 µL of n-hexadecane was quickly injected into the glass tube by syringe. Then analysis into a gas chromatograph. The extraction efficiency of amitriptyline and chlorpromazine from water sample was 71.34-73.52% and 73.83-91.09% was obtained. This method was effectively applied to the determination of the analytes in drinking water, tap water and lake water samples.

Hao et al. [43] studied the Extraction and Determination of Oleanolic Acid and Ursolic Acid in Chinese Medicinal Herbs by Dispersive Liquid-Liquid Micro-extraction Coupled with HPLC-UV. In this study, a process was developed by combining the HPLC-UV with DLLME for the determination and extraction of UA and OA in traditional Chinese medicinal herbs (CMHs). Amount of abstraction solvent, quantity of dispersive solvent, removal time, aqueous phase, pH, ionic strength, centrifugation time and speed, and sample volume Variables influenced was examined and optimized.

Guo et al. [44] studied the low-density solvent based/solvent demulsification DLLME coupled to gas chromatography−mass spectrometry (GC/MS) analyzed. The most substantial innovation of this technique is the computerization. The whole method, including the removal of the typical analysts (phthalate esters) by DLLME from the aqueous sample solution, breaking the emulsion after extraction, muster of the extract, and investigation of the extract by GC/MS, was totally automatic. The enrichment factor was obtained between 178 and 272 for the phthalate esters. This process was analyzed by GC/MS that can be worked automatically across multiple tests. **Farajzadeh et al. [45]** studied the first time prepared the extraction solvent lighter than water by DLLME method. The selected compounds were three organo phosphorus pesticides (OPPs) and the suggested technique was passed out for their preconcentration from water samples. Extraction was carried out by DLLME which is a combination of cyclohexane (extraction solvent) and acetone (disperser) was inserted into the liquefied sample by needle and then centrifuge. After centrifugation cyclohexane was collected at the upper side in the form of fine droplets. The top phase (0.40 lL) is inserted into the gas chromatograph (GC) for parting. For the detection of analytes, MS (for low concentrations) or flame ionization detector (FID) (for high concentrations) were used. Under the optimal conditions, the enrichment factors (EFs) reached from 100 to 150 and removal recoveries varied between 68% and 105%.

Jahromi et al. [46] studied the DLLME method was effectively used as a sample preparation process for graphite furnace atomic absorption spectrometry(GFAAS).in this technique, methanol (500µL, disperser solvent) contained carbon tetrachloride (34µL,extraction solvent) and ammonium pyrrolidine dithiocarbamate (0.00010g ,chelating agent) was speedily inserted into the sample of water covering cadmium ions (interest analyte) by needle then gloomy solution obtained as a result fine droplets carbon tetrachloride formed. Later the centrifugation for 2 min at 5000 rpm at the bottom of tube these drops were stabled. Then the analyte was determined by GF AAS.

Rezaee et al. [47] studied the Determination of organic compounds in water using dispersive liquid–liquid micro-extraction. In this method rapidly injected (8.0 µL C_2Cl_4) extraction solvent and (1.00 mL acetone) into sample by syringe, cloudy solution was obtained and then centrifuge.

After the centrifugation fine droplets of extraction solvents was collected at the bottom of centrifuge tube. The enrichment factor was 603. The capability of DLLME method in the removal of other organic mixtures such as organo chlorine insecticides, organo phosphorus pesticides and substituted benzene combinations from water sections were considered.

2.4 Deep eutectic mixture solvents

Due to the adverse effects of conventional solvents, now a days investigators have concerned more on the applications and discoveries of new green solvents. Due to special physical and chemical properties of Ionic liquids (ILs), it has attracted the attention as a green solvent [48].The properties high boiling point and low vapor pressure, which make easier to recycle, as a green solvents ILs were qualified. However many researcher have been reported out the ILs are very toxic, hazardous and have very poor biodegradability. ILs also required high purity since impurities, even in traces changes their physical properties, other the drawbacks of ILs, such as poisonousness and high price, limits the use of ionic liquids [49].To overcome the limitations associated with ILs, new green solvent emerged deep eutectic solvents (DESs). DESs, a new class of green solvent, which consisting the hydrogen bond donor HBD quaternary salt at room temperature, DESs green properties attract the considerable attention. DESs composed of a salt at room temperature (HBD) which melting point is lower than the individual components. Most of DESs are inexpensive, biodegradable and easy to prepare. The term “deep-eutectic solvent” (DES) was composed in 2003 by Abbott and co-workers [50]. They were formed the DES by the mixing of two high melting point solid martials.

A eutectic system is a combination of elements or chemical compounds that exhibits a single chemical composition that solidifies at a lower temperature than any other components. Figure 1.1, the eutectic point is given by the intersection of the eutectic composition and temperature.

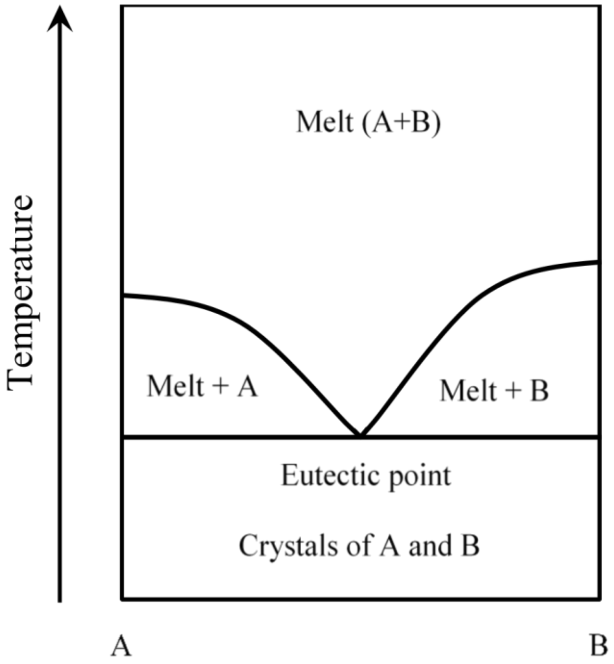


Figure 2.1. Schematic representation of two component phase diagram of eutectic point.

Various types of HBD and HBA are shown in figure2.2, which can be used for preparation of DESs. The resulting DESs are characterized by lower melting point than the individual component. [51, 52 and 53]. Most widely used component choline chloride (ChCl) are used in the formation of DESs because it is biodegradable, inexpensive and non-hazardous quaternary ammonium salt which can be either removed and synthesized from fossils reserves and biomass (million metric tons) by economical process.

In the combination of hydrogen bond donor (HBD) such as renewable carboxylic acids re (e.g. citric, oxalic, amino acids or succinic), urea or renewable polyols (e.g. carbohydrate , glycerol), ChCl are capable of forming DES .

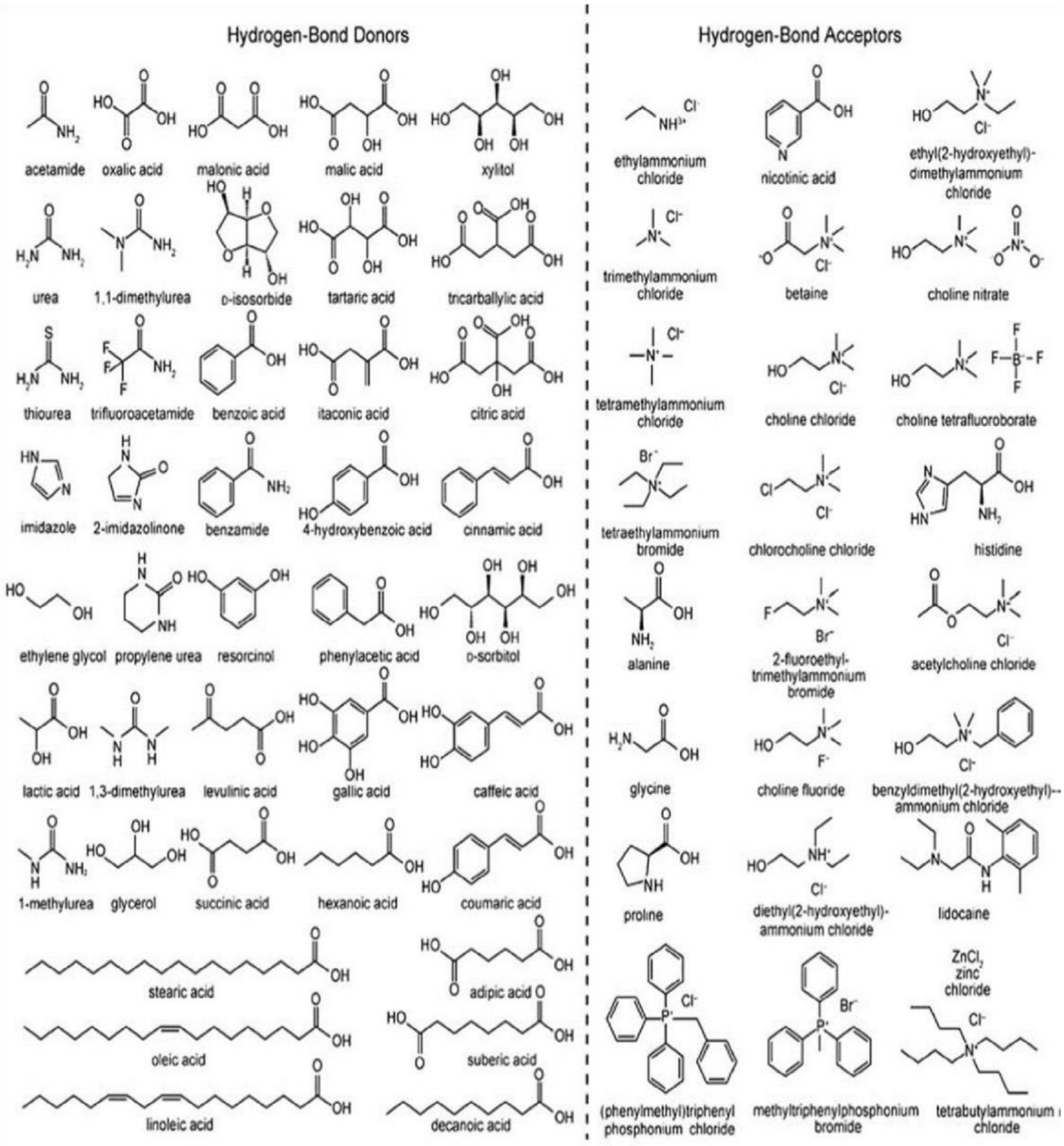


Figure 2.2 Certain HBD and HBA associates that can be combined to form a DES [51]

2.5 Advantages of DESs

DESs can be synthesized very economically, by using very simple method and at high purity due to low cost and the high purity of starting materials. The procedure is straight forward and very simple, by mixing only quaternary ammonium, sulfonium and phosphonium salt and HBDs in appropriate mole ratios. No waste products are formed during preparation of DESs. Therefore according to green chemistry, DESs synthesis is green and environmentally benign because zero emission during formation, zero E-factor value and excellent mass efficiency [54]. In economical terms DESs are less expensive than ILs. The major advantages of DESs are that they are designer solvents and their properties (physical, chemical and thermal) can be easily altered as per requirements by changing the components and their ratios [55].

DESs are composed of both the ionic and non-ionic species so DESs are not like as ionic liquids (ILs). The Physico-chemical properties of DESs (viscosity, conductivity, density, refractive index, surface tension, chemical inertness, etc.) are very near to ILs. DESs have several advantages over traditionally used ILs and other solvents like, non-toxicity, easy to prepare and low price. Since DESs are prepared by intercourse of two components simply, thus it by-passes the all difficulties which are produced into the ILs like purification and waste disposal. Most DESs are biodegradable chemical inertness with water (i.e. easy loading) and reinforcing the greenness of these media. DESs have several applications some of them are illustrated in Table 2.2.

Table 2.2 Applications of different deep eutectic solvents in various processes

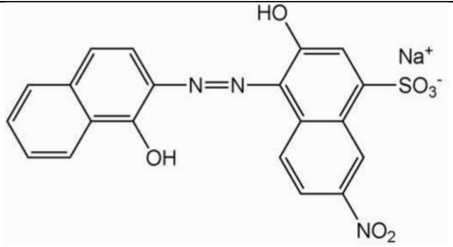
S. No.	DES	Ratio	Application	Reference
1	L:C	2:1	LC used for the separation of co_2 gas.	[57]
2	G:C	1:1	Aliphatic + ethanol separation	[58]
	L:C	2:1		
3	C:G	1:2	For the extraction of phenolic compounds from virgin olive oil (VOO).	[59]
	C:L	1:2		
	C:U	1:2		
	C:S	1:1		
	C:S	4:1		
	C:1,4-Butanediol	1:5		
	C: Xylitol			
	C:1,2-Propanediol	2:1		
	C: M	1:1		
		1:1		
4	C:G	1:2	Using DES grape skin phenolics are extracted	[60]
	C:O	1:1		
	C:M	1.5:1		
	C: Sorbose	1:1		
	C :Proline : M	1:1:1		

5	C: M TMAC: G TBAC:TEG PEG,PR	Mostly used 1:1 1:2	Used for extract the fuels by using desulfurization process.	[61]
6	THAB:GL THAB:EG	1:2 1:2	Used as a extracting agent for Separating the (hexane + benzene)mixture	[62]
7	CCL:U CCl:GL(best)	1:2 1:2	DES used for the separation of pyridine and toluene from n-hexane, and n-butanol from toluene	[63]
8	G:CCl G:TEA	4:1 2:2 4:1 2:1	Used for the separation of the azeotropic mixture {methyl ethyl ketone + ethanol}.	[64]
9	CCL:GL CCL:LA CCL:EG	1:2 1:2 1:2	liquid–liquid extractions of ethanol from the heptane + ethanol azeotropic.mixtures with the three DES	[65]
10	CCL:U CCl:EG CCl:MA	2:1 2:1 1:1	For determine the solubility of metal oxides.	[66]
11	CHAC:G, A or EG	1:2 1:1 2:1	Study their effects on enzyme activity and stability	[67]

2.6 Eriochrome Black-T (EBT) Dye

EBT is an azo dye which is generally known as indicator dye. IUPAC name is 1-[1-hydroxy2naphthol azo]-6-nitro -2-Naphthol- 4-sulphonic acid sodium salt. EBT has a high solubility in acidic organic solvents but less in water. Chemical EBT is a diprotic dye with pKa values of 6.6 and 11.6 over a pH range between 2.0 to 11.0. In its protonated form, the Erichrome black-t dye attains a blue color in a buffered solution of pH = 10 and turns red on forming a complex with calcium, magnesium, or other metal ions. It is a major group of azo dyestuffs, so extensively used in printing, leather and textile industries and contributes for more than 50% of the global dye production. However, it has found during research the anionic dyes effluents in the industry one of the problematic due to complex molecular structure towards water, light and chemicals.

Table: 2.3 Properties of EBT dye

Name	**Molecular weight**	**Molecular formula**	**λ_{max}**	**Chemical structure**
Eriochrome black-T dye	461.38	$C_{20}H_{12}N_3NaO_7S$	551 nm	

2.7 RESEARCH GAP

On the basis of literature discussed above, following research gaps have been identified

- Ionic liquids (ILs) have been used as novel extracting agent for dye removal from waste water. ILs are very expensive to prepare than other organic solvents and are toxic and non-biodegradable. DESs have similar properties as ILs and are very economical, and biodegradable. Unfortunately, no study is reported for dye extraction from water using any DES. However, only one study has been reported for metal extraction from water using DES.

- There is a need to explore the application of DES as solvent which are more suitable, economical and offer excellent properties like, environmental friendliness, biodegradability, nontoxic, water compatibility, recoverable and non-flammability.

3.1 General

In the current research, Liquid-Liquid Micro-Extraction technique has been implemented for dye extraction. The Eriochrome Black-T is selected as target pollutant to be removed from aqueous solution because of its complex molecular structures that is resistant towards biodegradation, thereby turning them into potential carcinogens and mutagens. The glycolic acid: choline chloride DES is employed for removal of this dye. DES is being used for the first time for dye extraction. Other than this it is biodegradable in nature and consumes only a small quantity, so it is considered to be a novel, reliable, potent, quick, eco-friendly and economical method for dye removal, thus offering high dye extraction efficiencies .

3.2. Instrumentation

A Perkin Elmer Lambda 35 UV-Vis Spectrophotometer with an absorption range between 200nm-700nm, a CF15RXII type Centrifuge (Hitachi Koki Co. Ltd., speed 300-15000 rpm, frequency 50/60 Hz, temperature range between -9 to 40 $^{\circ}$C, Time 5 sec to 99 min., rotor speed 3149 Rotational Centrifugal Force), graduated measuring cylinder, beakers (1000 mL), bottles (500 mL & 300 mL), glass cuvettes (Optiglass Ltd., T % > 80%, 10 mm path length), test tubes, hot air oven, glass pipette (10 mL & 2 mL), weighing balance (0.1-120 mg)), pH meter, round bottom centrifuge tubes (max. volume 10mL) . Magnetic stirrer with an adjustable rpm was purchased from Medi Lab Enterprises, Chandigarh.

3.3 Reagents and Dye solution

Glycolic acid (pure 98%) and Lactic acid (98%) were purchased from Alfa Aesar, England and choline chloride (>98%) were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Erichrome black-T dye (>99%) purchased from Loba Chemical Pvt. Ltd. Mumbai, India. Double distilled water (prepared in lab) was used for experimentation.

3.3.1 Eriochrome Black-T Dye

Erichrome Black-T dye appearance, physical state and purity at room temperature (2°C) are: EBT dye as Brown-black colored powder appears in solid state with 99% purity figure 3.1.

Figure 3.1: The Erichrome Black-T Dye

3.3.2 Dye stock solution

A stock solution (1 g/L) of Eriochrome Black-T dye was prepared by dissolving the appropriate quantity of the dye in double purified water. Working solutions of desired concentrations were prepared by appropriate dilution of the dye stock solution as shown in the figure below Figure.3.2.

Figure 3.2: Stock solution of Erichrome Black-T dye

3.4 Preparation of Deep eutectic mixture solvent

In this study for preparation of deep eutectic mixtures glycolic acid and choline chloride were used as hydrogen bond donor and hydrogen bond accepter respectively. Properties of selected HBD & HBA are shown in Table 3.1. DES was prepared using an electronic balance with precision of 0.02g, both the glycolic acid and choline chloride were added into a glass flask in predefined molar ratios (given in Table 3.2) and homogenously mixed before starting the heating. The temperature was controlled using a thermostatic oil bath. When the melting of mixture produce enough liquid, the magnetic stirring was initiated. DES in this study was prepared at 70 °C (±0.1 °C). The melting point of the mixture was always found much lower than the melting point of the starting materials. When the mixture formed a clear liquid, it was cooled down at room temperature. Prepared DES appears as a clear transparent liquid, without any characteristic odor. Density and viscosity of prepared DES was measured and water content was also measured using Karl–Fischer titration method.

Table: 3.1 Properties of HBD and HBA

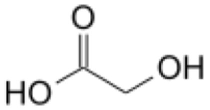
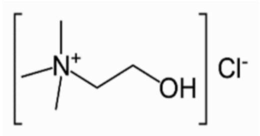
Name	**Glycolic Acid (HBD)**	**Choline Chloride (HBA)**
IUPAC Name	2-Hydroxyethanoic acid	2-hydroxy-N,N,N-trimethylethanaminium chloride
Molar mass	76.05 g/mol	139.62 g/mol
Chemical Formula	$C_2H_4O_7$	$C_5H_{14}ClNO$
Appearance	white, powdery solid	White or crystals(deliquescent)
Melting point	75 °C (167 °F; 348 K)	302°C (576 °F; 575 K) (decomposes)
Molecular structure		

Table 3.2: Deep eutectic solvents ratios

S. No	**Hydrogen bond donor**	**Hydrogen bond accepter**	**Molar ratio**
1	Glycolic Acid	Choline Chloride	1:1
2	Glycolic Acid	Choline Chloride	2:1
3	Glycolic Acid	Choline Chloride	3:1

3.5 Liquid-Liquid Micro-Extraction

LLME is a very rapid and less extracting agent (in the range of µL) consuming effective extraction technique. In this technique analytes are extracted using a small volume of a liquid through solvent micro-extraction (SME). The method is useful alternative for exploring the ability of an extracting agent due to its simplicity, effectiveness, low cost (minimal solvent use), and less time consumption. The extraction process was performed at room temperature (25 ± 2 °C) as follows:

A 10 ml narrowed–bottom plastic centrifuge tube carrying 6ml aqueous solution of EBT dye (in studied concentration). Afterwards, DESs (extracting solvent, varied in different concentration ranges) were introduced rapidly into the sample by using syringe. Consequently, a opaque solution was formed. The dyes were extracted into fine droplets. The mixture was then centrifuged for 4 min at 4000 rpm to reduce the sedimentation period. To allow proper partitioning of the dye and aqueous phase, the mixture was kept overnight. Upon observation, fine droplets of DES along with the extracted dye was found to be settled at the bottom of centrifuge tube, leaving the clear aqueous solution above them. Thus, two phases of DES+ Extracted dye and clear aqueous solution were distinctly visible.

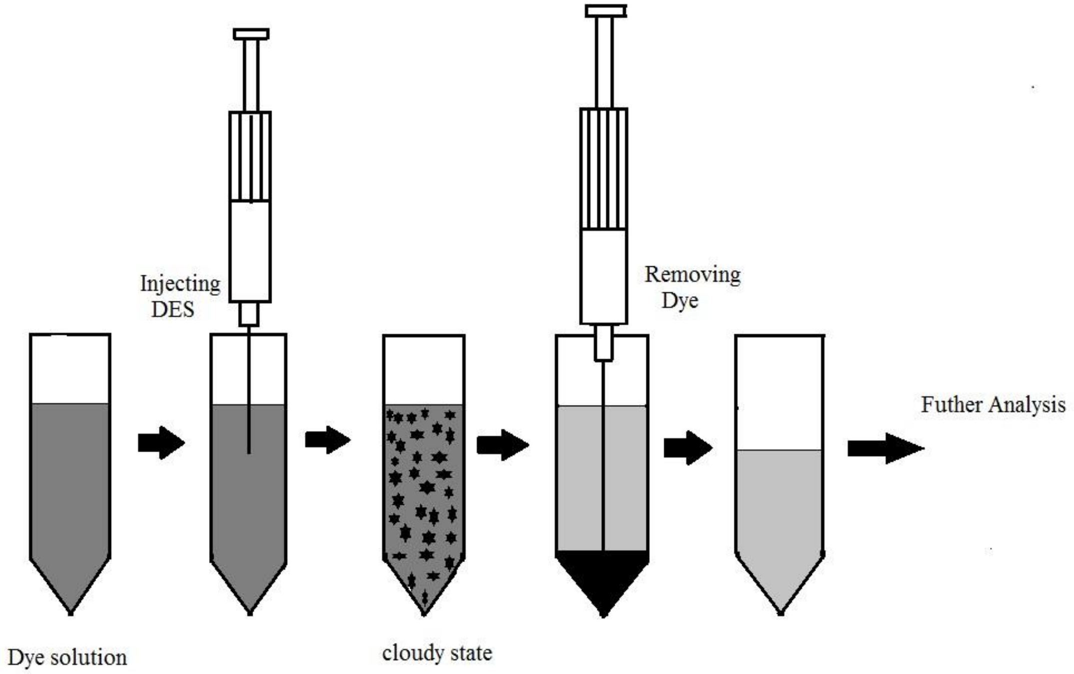


Figure 3.3: Schematic view of Liquid-Liquid micro-extraction technique

The remaining concentration (in mg) of dye left in the aqueous phase after the extraction process was determined by measuring the absorbance of aqueous phase in a UV-Vis Spectrophotometer with a wavelength range between 200-700 nm. Also, the absorbance of aqueous phase was measured thrice to maintain the accuracy. After that, the percentage extraction efficiency for dye was calculated by using equation 3.1. Pure solutions of dye concentration was initially calibrated in terms of absorbance values, which were recorded at the maximum wavelength $(\lambda)_{max.}$ = 551nm (Eriochrome Black-T).

$$\%\,Extraction\ \ Efficiency\ (E) = \frac{C_o - C_f}{C_o} \times 100 \qquad (3.1)$$

Where C_i and C_f represents initial and final dye concentrations in the aqueous phase respectively.

3.6 Physico-chemical properties of DESs

DESs are tailorable chemical solvents similar to ILs, prepared by combining the several quaternary ammonium salts (HBA) like, Choline chloride, tetra methyl ammonium chloride, tetra ethyl ammonium chloride etc. with different hydrogen bond donors properly. Different DESs show different physicochemical properties (density, viscosity, conductivity, pH and freezing point etc.) depending on their combination and ratios of HBD and HBA. The relevant physicochemical properties of DESs will be discussed in following section.

3.6.1 Density & Viscosity of DESs

The density and viscosity is one of the most significant physical properties for a solvent. Usually, densities of DESs are examined by measuring the specific gravity meter (Pyncometer) or specific gravity bottle. The density and viscosity data of prepared DESs at room temperature are listed in Chapter 4 (Table 4.2). Like Ionic liquids viscosity is a very important property for DESs also. In this study viscosities of prepared DESs were measured by Brookfield viscometer. Most of the DESs have high viscosity at room temperature (>100cP) except ChCl-Ethylene glycol based DES, due to the existence of wide hydrogen bonding linkage between the components, which results in a lower flexibility of free species inside the DES. The other factors like, large ion size, small void volume and other forces such as vander waals interaction or electrostatic force may affect the DES viscosity. Low viscosity of DES is highly required for their potential applications.

4.1 General

This chapter reports on Liquid–Liquid Micro-Extraction of indicator dyes namely Eriochrome Black-T dye from its aqueous solutions by using different DESs.

Several experiments on the extraction of EBT dye have been carried out with the prepared DESs for several process parameters stated in Table 4.1. The experimental analysis have been used to compute the performance of process in terms of extraction efficiency of different DESs.

Table 4.1: Process parameters

Parameter	Values
Dye concentration (mg/L)	50–500ppm
Amount of DES Liquid (µL)	10–500µL
pH	2–10
DESs [G:C]	(1:1, 2:1,3:1)

In general, the selection of an extracting agent is based on two objectives; it must remove analytes of concern efficiently as well as it should meet the process economics. To make the process economical, the selected extracting agent has to be more immiscible in the sample solution, for reducing the consumption of extraction solvent. Due to the requirement of this characteristic, DES has been selected as extracting agents for this study.

4.2 Calibration Curve of studied dye

Before the samples can be analyzed, a standard calibration plot must be produced. To produce the standard calibration plot, samples of known concentration (0 to 100 mg/L) were prepared and absorbance were measured at λ_{max} = 551 nm and a standard calibration curve of absorbance versus Concentration was plotted (Figure 4.1).

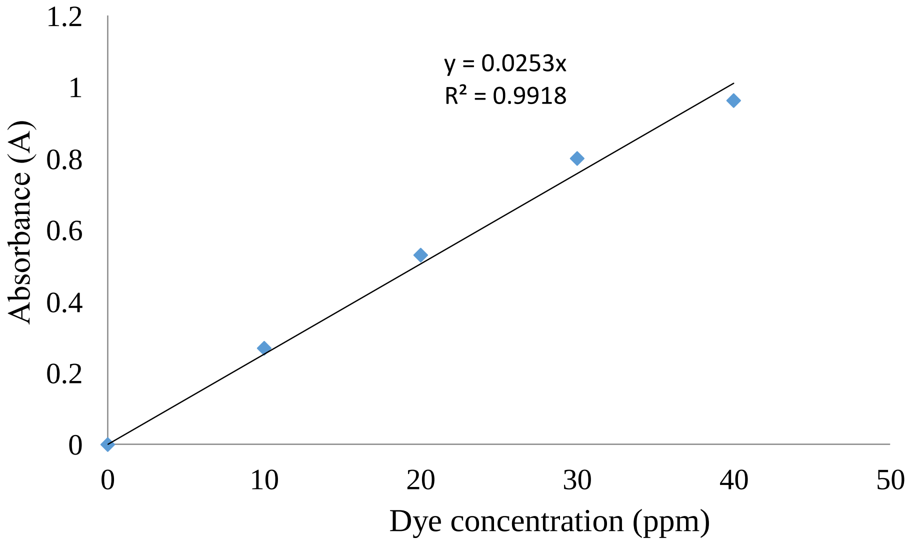


Figure 4.1: Standard Calibration Curve for Eriochrome Black-T dye

Physical properties like density and viscosity plays an important role in extraction process. The prepared DESs [G:C] of different compositions (1:1, 2:1, 3:1) were analyzed for its physical properties and are reported in table 4.1.

Table 4.2 Density and viscosity of DESs.

Sr.No	Solvent	Density (g/cm$^{3)}$	Viscosity (cP)	Moisture content (%)
1	G:C [1:1]	1.3078	398.77	1.55
2	G:C [2:1]	1.380	263.2	1.45
3	G:C [3:1]	1.4166	225.2	1.2

The performance of different EDSs on extraction of EBT is reported in terms of extraction efficiency. The effect of different process parameters like; pH, DES dose and initial dye concentration has been studied on extraction efficiency.

4.3 Effect of pH on dye extraction efficiency

Previous researches on dye extraction reports that the pH of aqueous dye solution significantly changes the extraction efficiency by affecting the degree of ionization of a dye molecule. In this study, effect of pH on extraction efficiency was studied and optimized. The pH study range was selected between 2–10 at C_o=200 mg/L (EBT dye initial concentration). To adjust the pH, HCl and NaOH solutions were used. The prepared DESs [G:C] of three different compositions (1:1, 2:1, 3:1) were used with dosage of 200 µL. Figure 4.2 represents the effect of pH on dye extraction efficiency using different DESs.

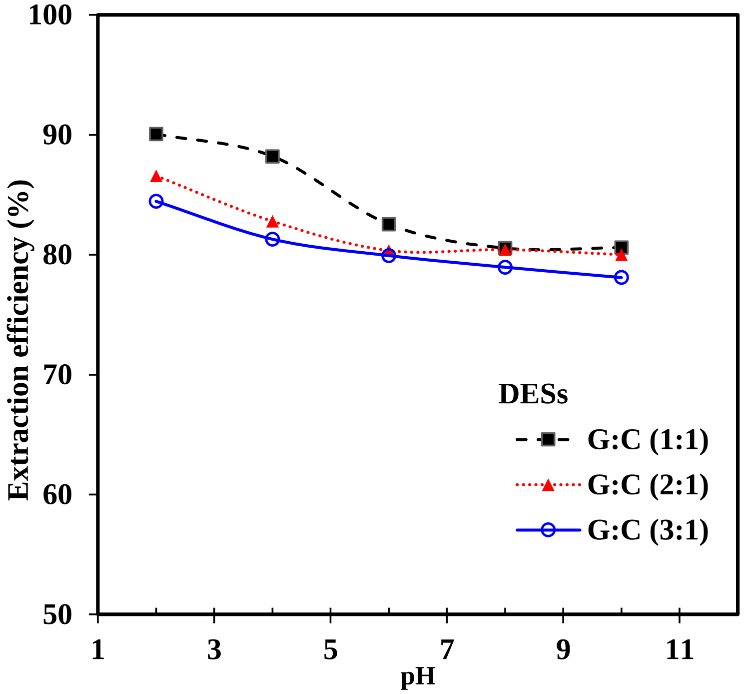


Figure 4.2 Effect of pH on dye extraction efficiency by using DESs

It can be seen from the figure 4.2 for G:C (1:1) that, as pH of dye solution increases from pH=2, extraction efficiency decreases up to pH=6. For all pH>6 extraction efficiency becomes almost constant to ≈81%. Maximum extraction efficiency is achieved at lowest studied pH (2) concluding pH=2 as optimum pH. Similar trend were also observed for all other DESs studied G:C (2:1) and (3:1). Although G:C [1:1] shows maximum efficiency ≈ 90% amongst all three DESs studied. Further, G:C (3:1) showed minimum extraction efficiency amongst all DESs, showing ≈81% extraction efficiency at optimum pH (2).

This behavior of the dyes on the extraction efficiency is explained by the degree of ionization and charged state, and hydrogen bonding, which depend on the pH. The sulfonate group of EBT dye (anionic dye) turn out to be anionic in the aqueous solution, and decrease in pH of dye aqueous solution turn azo group to be protonated, therefore, dye molecule becomes zwitterionic (67). Moreover, anionic form of dye molecule dominates in pH> pKa (pKa value for EBT 6.2) and zwitterionic at pH< pKa. At low pH, the solution becomes hydrogen ion rich (positively charge), and zwitterionic form of dye molecule dominates which establishes the strong hydrogen bonding between OH group of EBT dye and Cl^- of DES, which in turn increases dye extraction efficiency. Similarly at high pH, anionic form of dye molecule dominates which decreases which create the weak hydrogen bonding and the extraction efficiency decreases.

Therefore, it can be concluded that EBT dye molecule in zwitterionic form prefer to be more partitioned in DES as compared to water, while in anionic form in water.

4.4 Effect of DES Dose

To examine the effect of extracting solvent (DES) dose, different volumes (10-500 µL) of extraction solvent (DESs) were considered and its effect on extraction efficiency was studied.

Various dosage (10, 20, 30, 40, 50, 100, 200, 300, 400, 500 µL) of different DESs [G:C] (1:1, 2:1, 3:1) were introduced into the 10 ml dye solution having C_o=200 mg/L and pH=2 for this study. Figure 4.3 show the effect of DES dose on extraction efficiency of EBT dye.

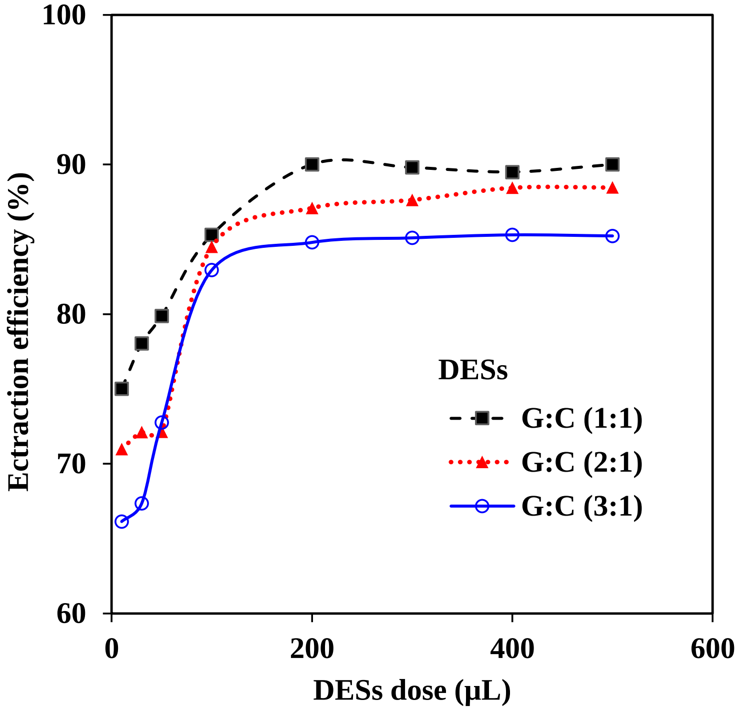


Figure 4.3 Effect of volume of DESs on dye extraction efficiency

It can be seen from figure 4.3 that as the amount of DESs [G:C(1:1,2:1,3:1)] increases extraction efficiency increases till 200 µL. Further increment of DES amount beyond equilibrium, extraction efficiency remains almost constant, implying that the extraction process is limited by the limiting DES dose. This trend was followed by all the three DESs [G:C(1:1,2:1,3:1)] studied. Increasing DES dose increases the availability of OH and Cl^- groups which in turn facilitates the hydrogen bonding between OH group of EBT dye and Cl^- of DES. It can also be observed that, at lower dosage the DESs (G:C) performance order is 1:1>2:1>3:1. However, at higher dosage the extraction efficiency observed nearly same (90%).

Therefore, it may be concluded that the DES G:C (1:1) with dose of 200µL is performing better than the other DESs G:C (2:1, 3:1).

4.5 Effect on initial dye concentration

Effect of EBT initial dye concentration (C_0) on extraction efficiency value was studied at varying C_o (50, 100, 200, 300, 400 and 500 ppm) at pH=2.0 and DESs dosage of 200 µL, respectively, and presented in Figure 4.4.

It was observed that, as the C_o increases, extraction efficiency value of EBT dye increases up to C_0= 300 mg/L, and for all C_0 >300 mg/L change in E value was marginal. This was observed true for all the three DESs [G:C (1:1,2:1,3:1)]) studied (Figure 4.4). Furthermore, for EBT dye extraction, DES G:C (1:1) performed best showing the highest extraction efficiency value at all the C_0 studied with maximum E value of ≈90%.

Extraction process is limited by limiting analyte (dye in this case) concentration and/or by limiting extraction solvent dose. Increasing extraction efficiency value with C_o up to C_o =300 mg/l, reveals that the DESs possess high extraction capacity of extracting more dye from the aqueous phase, but the process is in equilibrium with available extractable dye from the aqueous phase, and the extraction process is limited due to the limiting dye concentration. For all C_0 >300 mg/l the extraction efficiency values are nearly constant, due to the fact that extraction process is limited by limiting extraction solvent dose.

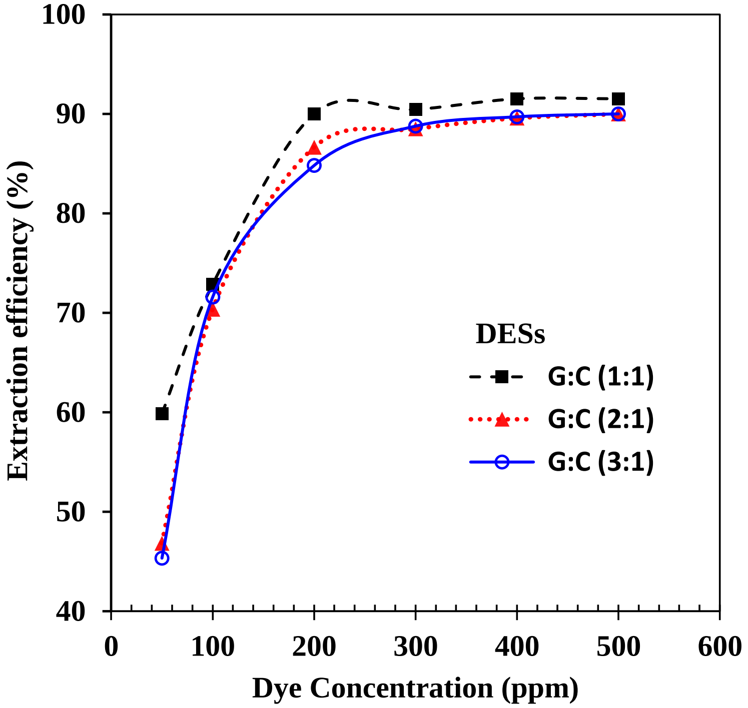


Figure 4.4 Effect of concentration of dye on extraction efficiency.

The extraction of EBT dye on prepared DESs is expected to be due to the hydrogen bonding between EBT dye molecule and DESs. This can be confirmed by the FTIR and NMR analysis. FTIR is especially used to identify the functional groups and interaction of the hydrogen bonding [67]. As shown in fig 4.5, peaks at 3301 cm^{-1} formed from the stretching vibration of O-H functional groups. The FTIR spectra of DES [GC (1:1)], blank and EBT dye loaded (after extraction of EBT on DES), are shown in Figure 4.5. The FTIR spectrum of the blank DES is conquered by a band near 1734 cm^{-1} with a number of other somewhat weaker bands near (1085 and 1182) cm^{-1}. The band near 1734 cm^{-1} can be assigned to “free” carbonyl group in glycolic acid, whereas the OH group is hydrogen bonded to the Cl^{-} anion of choline chloride (68). The band near 1182 cm^{-1} can be assigned to C– N stretching in choline chloride. Broad band appeared at higher wavenumbers, near 3301 cm^{-1}, indicating OH stretching region indicate the presence of multiple hydrogen bonds in the form of OH (glycolic acid)···Cl^{-}, OH(choline)···Cl^{-}, and glycolic acid– glycolic acid hydrogen bonds (68). Loaded DES with EBT dye shows the shifting of peaks from 3301 to 3338 cm^{-1} and 1734 to 1637 cm^{-1} with increased and decreased intensity, respectively. This indicate that the functional groups at these wave number participate in the extraction of EBT dye. As discussed above, the strong peaks at these bands (3301 and 1734 cm^{-1}) are associated with

OH stretching with multiple hydrogen bonds. Therefore, shifting and variation in peak intensities confirms the involvement of hydrogen bonds between OH (EBT dye) and OH and /or Cl^- of DESs in the extraction of EBT dye on DESs.

^{1}H NMR spectra for blank and EBT dye loaded DES are shown in Figure 4.6. ^{1}H NMR spectra of blank DES showed that the DES was successfully prepared with some impurities (marked peaks in spectrum) of possibly of esters resulting due to reaction between glycolic acid and choline chloride (69). Dye loaded spectra of DES shows shifting of peaks with some new peaks confirming the EBT dye extraction on DES.

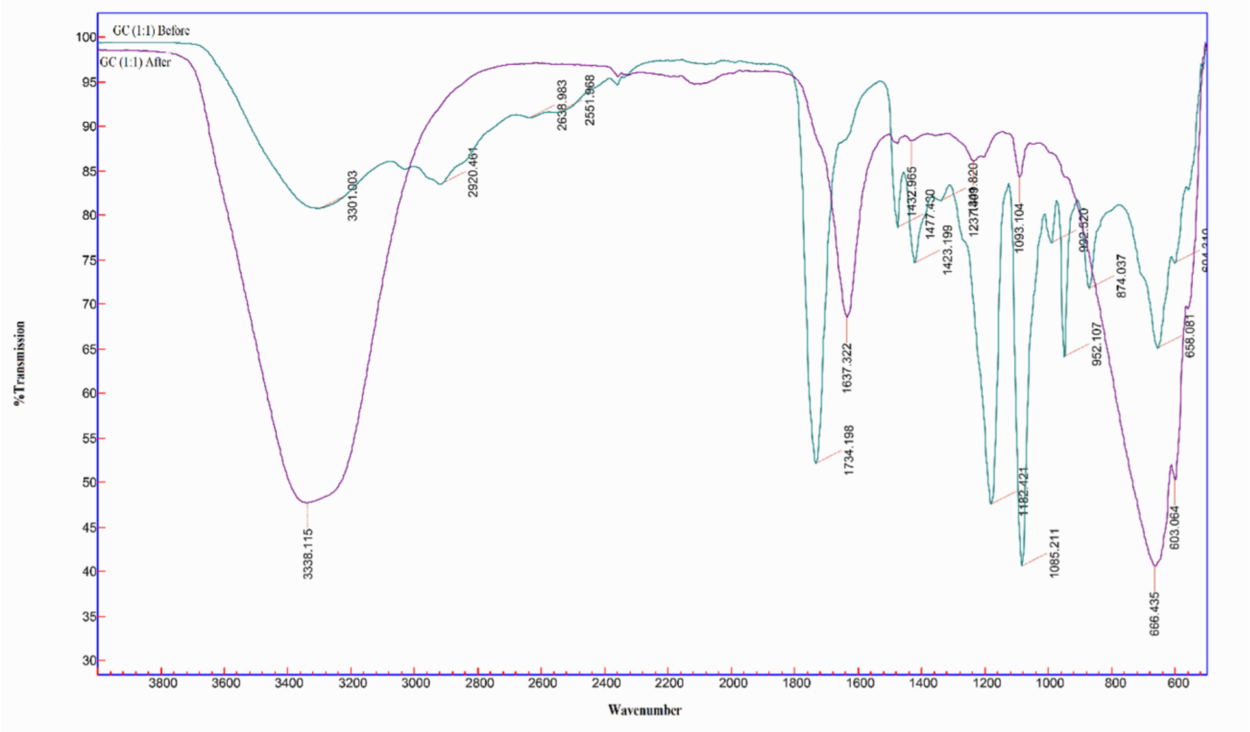


Figure. 4.5. FTIR spectra for DES GC (1:1) before extraction (upper spectrum) and after extraction (lower spectrum)

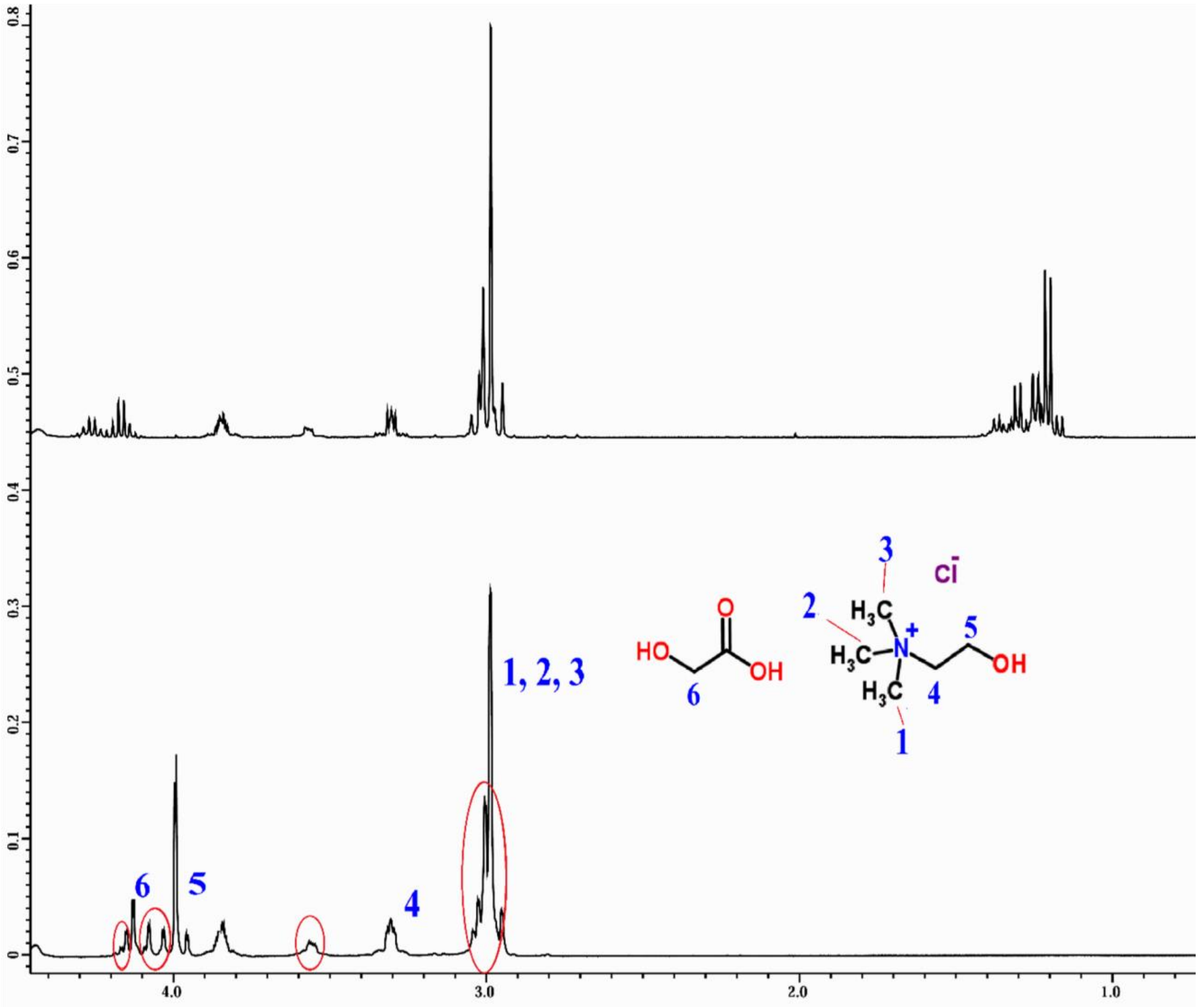


Figure. 4.6. ^{1}H NMR spectra for DES GC (1:1) before extraction (lower spectrum) and after extraction (upper spectrum)

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

In this study, glycolic acid and choline chloride based DESs were introduced for the first time as an extracting solvent for dye removal. LLME (liquid-liquid micro-extraction) method was adapted for evaluating the performance of selected DESs. DESs showed capability to extract dyes from water, depending on the structure of dye and bonding between dye and selected DES. On the basis of performance of above mentioned DESs on extraction ability of EBT dye, following conclusions were drawn.

- pH of the solution showed significant effect on extraction efficiency. Maximum extraction efficiency of ≈ 90% was achieved at lowest studied pH (2) for DES G:C [1:1], while G:C (3:1) showed minimum extraction efficiency amongst all DESs, showing ≈81% extraction efficiency at optimum pH (2).
- It was found that EBT dye molecule in zwitterionic form prefer to be more partitioned in DES as compared to water, while in anionic form in water.
- Dose study revealed that the extraction process is limited by the limiting DES dose.
- It was observed that, at lower dosage the DESs (G:C) performance order is 1:1>2:1>3:1. However, at higher dosage the extraction efficiency observed nearly same (90%).
- Increasing extraction efficiency value with C_o up to C_o =300 mg/l, revealed that the DESs possess high extraction capacity of extracting more dye from the aqueous phase, and the extraction process is limited due to the limiting dye concentration. For all C_0 >300 mg/l the extraction efficiency values are nearly constant showing extraction process is limited by limiting extraction solvent dose.
- FTIR and NMR studies revealed the involvement of hydrogen bonds between OH (EBT dye) and OH and /or Cl^- of DESs in the extraction of EBT dye on DESs.

5.2 RECOMMENDATIONS

From the above conclusions, the following studies are recommended in further study:

- It is recommended that extraction ability of DESs should also be explored for other hazardous dyes.
- There is a need to investigate the regeneration possibility of used DESs by suitable method.
- DESs based on different HBD and HBA should also be used for extraction of different dyes.
- Pilot-scale study is recommended before commercial exploitation of DES as extracting agent for dye extraction.

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