CHAPTER III

EXPERIMENTAL SECTION

STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES, PURIFICATION AND APPLICATIONS OF THE CHEMICALS USED IN THE RESEARCH WORK

III.1.1. SOLVENTS

The detailed information of the aqueous and non-aqueous solvents used in the research work are given below:

III.1.1.1. Water (H_2O):

Source: Distilled water, distilled by fractional distillation method in Lab.

Purification: Water was first deionised and then distilled using alkaline $KMnO_4$ solution to remove any organic components present there.

Water	
Physical state	Liquid
Molecular Formula	H_20
Molecular Weight	18.02 g·mol^{-1}
Density	0.99713 g·cm^3
Viscosity	0.891 mP·s

Application:

Water is the universal solvent. A greater number of substances dissolve in it than in any other liquid. Chemical operations are frequently carried on in solution, that is to say, the substances which are to act chemically upon each other are first dissolved in water. The object of this is to get the substances into as close contact as possible. If we rub two solids together, the particles remain slightly separated, no matter how finely the mixture may be powdered. If, however, the substances are dissolved and the solutions poured together, the particles of the liquid move so freely among each other that the come in dired contact, thus aiding chemical action. In some cases substances which do not act on each other at all when brought together in dry condition, act readily when brought together in solution **.**

III.1.1.2. Ethanol (C_2H_5OH):

Source: Purchased from Sigma Aldrich

Purification: Used as purchased.

Ethanol	
Physical state	Liquid
Molecular Formula	C_2H_5OH
Molecular Weight	46.07 g·mol^{-1}
Density	0.8029 g·cm^3
Viscosity	1.040 mP·s
CAS No	64-17-5

***Application**:*

Ethanol is used as a solvent, and as an antifreeze in pipelines. In some waste water treatment plants, a small amount of ethanol is added to waste water to provide a food source of carbon for the denitrifying bacteria, which converts nitrates to nitrogen to reduce the denitrification of sensitive aquifers. Ethanol is used on a limited basis to fuel internal combustion engines.

III.1.1.3. Methanol (CH_3OH)

Source: Purchased from Sigma Aldrich

Purification: Used as purchased.

Methanol	
Physical state	Liquid
Molecular Formula	CH_3OH
Molecular Weight	32.04 g·mol^{-1}
Density	0.792 g/cm^3
Viscosity	0.545 mPa·s
CAS No	67-56-1

Applications

Methanol is a traditional denaturant for ethanol and it is quite well known as "denatured alcohol" or "methylated spirit". Methanol is used by municipal and private treatment facilities to clean savage water, by eliminating harmful nitrates. It is used to produce dimethyl ether (DME), a clean-burning fuel, which is blended with liquefied petroleum gas (LPG) to act as an alternative home heating fuel. It can also be used directly as a diesel fuel replacement.

III.1.1.4. Acetonitrile (CH_3CN)

Source: Purchased from Sigma Aldrich

Purification: Used as purchased.

Acetonitrile	
Physical state	Liquid
Molecular Formula	CH_3CN
Molecular Weight	41.05 g·mol^{-1}
Density	0.786 g/cm^3
CAS No	75-05-8

Applications

Acetonitrile is mainly used in purification of butadiene in refineries. It is also used in battery industries because of having relatively high dielectric constant. Its ability to dissolve other electrolytes, low viscosity, low chemical reactivity makes it a good choice for both cyclic voltametry and high-performance liquid chromatography (HPLC).

Iii.1.2 Electrolytes And Non-Electrolytes

The electrolytes ionic liquids, and non-electrolytes amino acids, respectively, and other chemicals than these two categories that are used in the research work have been describing follow:

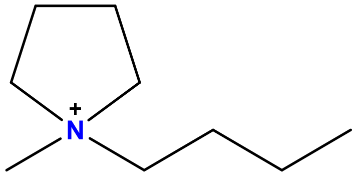
III.1.2.1. Ionic Liquids

1-butyl-1-methylpyrrolidinium chloride ([bmp]Cl)

Source: Purchased from Sigma Aldrich

Purification: Used as purchased.   .

Structure	**1-butyl-1-methylpyrrolidinium chloride ([bmp]Cl)**	
	Physical state	Liquid
	Molecular Formula	$C_9H_{20}ClN$
	Molecular Weight	177.71 g·mol^{-1}

	CAS No	479500-35-1

Application

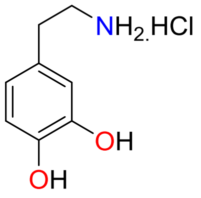
Ionic liquids have wide range applications such as the recovery of [96]as potential lubricants, in solar cells [97], for heat storage [98], in nuclear fuel processing, as sol-gel templates [98], and in the dissolution of cellulose [99]. Ionic liquids are also being used in tissue preservation.

III.1.2.2 Drug molecules

1. Dopamine Hydrochloride

Source: Purchased from Sigma Aldrich .

Purification: Used as purchased

Structure	**Dopamine Hydrochloride**	
	Physical state	Solid
	Molecular Formula	$(HO)_2C_6H_3CH_2CH_2NH_2 \cdot HCl$
	Molecular Weight	189.64 g·mol^{-1}
	CAS No	62-31-7

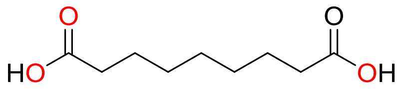
Application

Dopamine is considered as a significant neurotransmitter (NT) in the mammalian central nervous system from the catecholamine family [100]. This is also believed to have influences on the pathogenesis of some neurodegenerative disorders such as Alzheimer and Parkinson diseases [101] [102].

2. Azelaic Acid

Source: Purchased from Sigma Aldrich

Purification: Used as purchased

Structure	Azelaic Acid	
	Physical state	Solid
	Molecular Formula	$HO_2C(CH_2)_7CO_2H$
	Molecular Weight	188.22 g·mol^{-1}
	CAS No	123-99-9

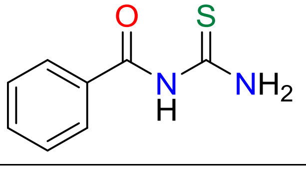
Application

Azelaic acid has anti-inflammatory, antibacterial, and keratolytic effects. Azelaic acid is widely used to treat a number of dermatoses and is universally accepted as an acne treatment [103, 104]. The compound has broad-spectrum bactericidal activity in vitro [105], which can be greatly enhanced by reducing the pH of the medium.

3. N-Benzoylthiourea

Source: Purchased from TCI, India .

Purification: Used as purchased

Structure	N-Benzoylthiourea	
	Physical state	Solid
	Molecular Formula	$C_8H_8N_2OS$
	Molecular Weight	180.23g·mol^{-1}
	CAS No	614-23-3

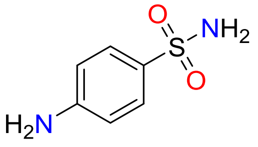
Application

NBTU can be used to control of animal pathogenic bacteria but also they have been shown to possess antitubercular, antifungal, antithyroid, insecticidal, anthelmintic and rodenticidal properties [106].

4. Sulphanilamide

Source: Purchased from Sigma Aldrich .

Purification: Used as purchased

Structure	Sulphanilamide	
	Physical state	Solid
	Molecular Formula	$H_2NC_6H_4SO_2NH_2$
	Molecular Weight	172.20 g·mol^{-1}
	CAS No	63-74-1

Application

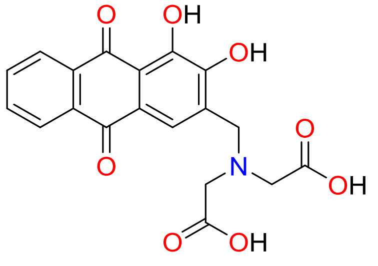
This compound has both antibacterial and antimicrobial properties and is used in the treatment of topical and internal infections. It can be found in medications for vaginal and urinary tract infections, pneumonia and bowel related issues. It inhibits the growth of yeast (fungus) and thereby decreases chances of infections [107] [108].

III.1.2.3 Dye molecule

1. Alizarin-3-methyliminodiacetic acid

Source: Purchased from Sigma Aldrich .

Purification: Used as purchased

Structure	Alizarin-3-methyliminodiacetic acid	
	Physical state	Solid
	Molecular Formula	$HO_2C(CH_2)_7CO_2H$
	Molecular Weight	188.22 g·mol^{-1}
	CAS No	123-99-9

Application

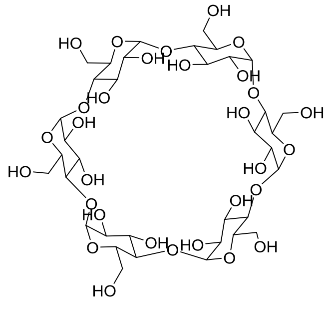
It is extensively used in textile industries, printing and paper industries. Alizarin complexone (AC) is a very well known reagent for the spectrophotometric determination of metals and it can form deeply coloured metal complexes [109].

III.1.2.3. Host Molecules

1. α-Cyclodextrin

Source: Sigma Aldrich, Germany

Purification*:* Used as purchased (w≥99.98%)

Structure	α-Cyclodextrin	
	Physical state	Solid
	Molecular Formula	$C_{36}H_{60}O_{30}$
	Molecular Weight	972.84 g·mol^{-1}
	CAS No	10016-20-3

Application:

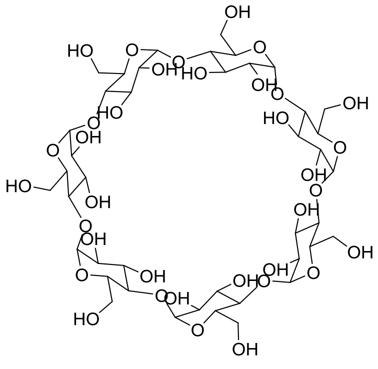
α-Cyclodextrin has widespread application in the area of medicine, food and cosmetics. During medicine manufacture, it can modify the persistence of medicine without being oxidized. In food industry, a-cyclodextrin is being used as a carrier and stabilizer for bulky guests for proper encapsulation. For example, cinnamic acid (CA)is a naturally occurring organic acid and is often found in fruits and spices having antimicrobial activity against pathogenic bacteria. This acid is sparingly soluble in water that limits its use. α-Cyclodextrin-Cinnamic acid inclusion complex is capable of reducing E. coli populations significantly due to its enhanced solubility in water [110]. It has been discovered that α-Cyclodextrin is very effective to solubilize free fatty acids . It can also be used as browning inhibitors in various fruit juices [111].Because of having smallest internal cavity among all cyclodextrins, the application of α-cyclodextrin-assisted molecular encapsulation in food industry and medicine is significantly limited.

The α-Cyclodextrin -fat complex was found to be resistant to normal lipolytic hydrolysis by lipases. Hence, α -cyclodextrin decreases the rate of absorption and bioavailability of dietary fat, which makes it usage as a weight loss supplement [112].

2. β-Cyclodextrin (β-CD):

Source: Sigma Aldrich, Germany

Purification: Used as purchased (w≥99.98%).

Structure	β-Cyclodextrin	
	Physical state	Solid
	Molecular Formula	$C_{42}H_{70}O_{35}$
	Molecular Weight	1134.98 g·mol^{-1}
	CAS No	7585-39-9

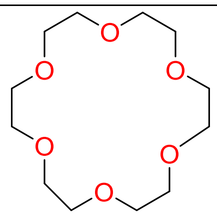
Application:

There is a no of research articles which describe the influence of β-CDs on dyeing. It has been described in literature that β-CDs can absorb dyes[113]and hence it can be used to eliminate loss of dye in waste water. It is also capable of improving dye uniformity and preventing the running of dyes during washing. β-CDs can form inclusion complex with aroma molecules by reducing their vapour pressure and it reflects in delay the breakdown of the molecules because of photo degradation. From literature survey we can find that, on using certain grafting agents with cyclodextrins (variables are the extent of grafting, nature of native and modified cyclodextrins and shapes and sizes of the substrate and guest molecule) makes the fabrics to retain fragrances for longer time [114]. β-CD cavities can trap bad odours and these cavities can be emptied during the washing process. Wang et al. [115]incorporated an antimicrobial agent miconazole nitrate into β-CD and applied it to fabric, which resulted in modification of antimicrobial properties.

3. 18-crown-6 (18C6):

Source: Sigma Aldrich, Germany

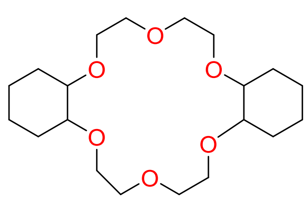
Purification: Used as purchased (w≥99.0%).

Structure	18-crown-6	
	Physical state	Solid
	Molecular Formula	$C_{12}H_{24}O_6$
	Molecular Weight	264.32 g·mol^{-1}
	CAS No	17455-13-9

4. Dicyclohexano 18-crown-6 (DC18C6):

Source: Sigma Aldrich, Germany

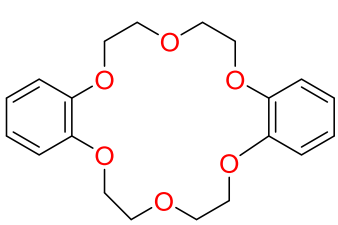
Purification: Used as purchased (w≥99.0%).

Structure	Dicyclohexano 18-crown-6	
	Physical state	Solid
	Molecular Formula	$C_{20}H_{36}O_6$
	Molecular Weight	372.50 g·mol^{-1}
	CAS No	16069-36-6

5. Dibenzo 18-crown-6 (DB18C6):

Source: Sigma Aldrich, Germany

Purification: Used as purchased (w≥99.0%).

Structure	Dibenzo 18-crown-6	
	Physical state	Solid
	Molecular Formula	$C_{20}H_{24}O_6$
	Molecular Weight	360.40 g·mol^{-1}
	CAS No	14187-32-7

Applications

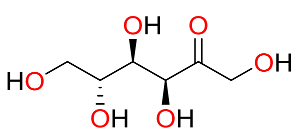
In the field of inclusion compounds, crown ethers (where the crown acts as 'host') is a key factor to track the movement of essential elements in the body such as enzyme's functions, which can be applied to develop new compounds for pharmaceutical usage. Crown ethers are well accepted as drug carriers. They also have medical applications as diagnostic or therapeutic agents [116].

III.1.2.4. Compounds used in Solution Chemistry

1. D(-) Fructose

Source: Thomas Baker, India.

Purification: Used as purchased (w≥99%).

Structure	D(-) Fructose	
	Physical state	Solid
	Molecular Formula	$C_6H_{12}O_6$
	Molecular Weight	180.16 g·mol^{-1}
	CAS No	57-48-7

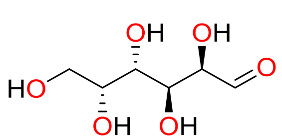
Application

Fructose is used in the formulation of sweet and beverages because of its sweetness. It is well known for enhancing taste of food in food industries. It is used in preperation of soft drinks, energy drinks, low-calorie products, etc [117].

2. D(+) Galactose

Source: Thomas Baker, India.

Purification: Used as purchased (w≥99%).

Structure	D(+) Galactose	
	Physical state	Solid
	Molecular Formula	$C_6H_{12}O_6$
	Molecular Weight	180.16 g·mol^{-1}
	CAS No	3646-73-9

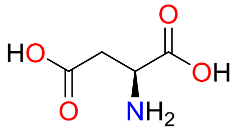
Applications

Galactose is considered a key source of energy and a essential structural element in complex molecules – is specifically important for early human development. In recent times, galactose has been reported as beneficial for a number of chronic neurological diseases. Galactose is much needed for human metabolism, it also plays significant role in energy delivery and galactosylation of complex molecules [118].

3. L-Aspartic acid

Source: Sigma Aldrich, Germany

Purification: Used as purchased (w≥98%).

Structure	L-Aspartic acid	
	Physical state	Solid
	Molecular Formula	$HO_2CCH_2CH(NH_2)CO_2H$
	Molecular Weight	133.10 g·mol^{-1}
	CAS No	56-84-8

Applications

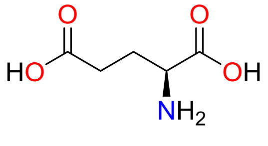
This amino acid plays an important role in the urea cycle and DNA metabolism and acts as a major excitatory neurotransmitter, which is sometimes found to be increased in epileptic and stroke patients

It has a special property to get racemized from L- to D-form, which makes it able for determining age of living and non-living systems [119].

3. L-Glutamic acid

Source: Sigma Aldrich, Germany

Purification: Used as purchased (w≥99%).

Structure	L-Glutamic acid	
	Physical state	Solid
	Molecular Formula	$HO_2CCH_2CH_2CH(NH_2)CO_2H$
	Molecular Weight	147.13 g·mol^{-1}
	CAS No	56-86-0

Applications

It acts as an excitatory neurotransmitter in the central nervous system of mammals [120]. It can also be served as a precursor molecule for the synthesis of various metabolites including N-acetyl-L-glutamate, δ-1-Pyrroline-5-carboxylate, β-citryl glutamate, L-γ-glutamyl-Lcysteine[121].

III. 2 EXPERIMENTAL METHODS

III.2.1 Preparation Of Solutions

A stock solution for each sample was prepared by mass (digital electronic analytical balance, Mettler Toledo, AG 285, Switzerland), and the required solutions were prepared by mass dilution of the stock solution. The uncertainty of concentration (molarity or molality) of the solutions prepared was found to be ±0.0002.

Iii.2.3. Measurements Of Experimental Properties

III.2.3.1. Mass Measurement

Using digital electronic analytical balance Mettler Toledo, AG 285, Switzerland mass in different cases were measured. The weighing pan of a high precision (0.0001g) is inside a transparent enclosure . Solutions were prepared by weight precise to ± 0.02 %. The weights were taken on a Mettler electronic analytical balance (AG 285, Switzerland).

Instrument Specification:

Readability	: 0.1 mg/ 0.01mg
Maximum capacity	: 210 g/81g/41g
Repeatability	: 0.1 mg/ 0.05 mg
Stabilization time	: 3 s/ 15 s
Adjustment with external weights	:200 g
Sensitivity	: ±0.003%
Display	: LCD
Interface	: Local CAN universal interface
Weighing	: Φ 85 mm, stainless steel
Effective height above pan	: 240 mm
Dimensions(w/d/h)	: 205×330×310 mm
Net weight /with packaging	: 4.9 kg/7.25 kg

III.2.3.2. Conductivity Measurement

Conductivity measurement was performed in Systronics Conductivity TDS meter-308. It can provide both automatic and manual temperature compensation. The conductance measurements were carried out on this conductivity bridge of accuracy ±0.01%, a dip-type immersion conductivity cell was used. Measurements were made in a thermostate water bath maintained at T = (298.15 ± 0.01) K. The cell constant was measured based on 0.01 M aqueous KCl solution. During the conductance measurements, cell constant was maintained within the range 1.10–1.12 cm^{-1}The measurements were made in a thermostatic water bath maintained at the required temperature with an accuracy of ± 0.01 K .

Instrument Specifications:

Frequency	100 Hz or 1 KHz Automatic
Range	0.1 µS to 100 mS. (6 decadic range)
Accuracy	±1% of F.S. ±1 digit
Resolution	0.001 µs
Range	0.1 ppm to 100 ppt. (6 decadic range)
Cell Constant	Acceptable. from 0.1 to 5.0
Power	230V AC, ±10%, 50 Hz
Dimensions	250(W)× 205(D)× 75(H)
Weight	1.25 Kg (Approx.)
Accessories	(i) Conductivity cell, cell constant 0.1 (ii) Conductivity cell, cell constant 1.0 (iii) Temp. Probe (PT-100 sensor) (iv) Stand & Clamp

III.2.4.3. Density Measurement

The density measurement was performed with the help of Anton Paar DMA 4500M digital density-meter with a precision of ±0.0005 $g \cdot cm^{-3}$.

In the digital density meter, the mechanic oscillation of the U-tube is e.g. electromagnetically transformed into an alternating voltage of the same frequency. The period τ can be calculated using the following equation

$$\rho = A \cdot \tau^2 - B \qquad \text{(III.1)}$$

A and B are instrument constants of each oscillator and ρ is the density of the sample.

Instrument Specification:

Density	0 to 1.5 g.cm^{-3}
Temperature	15^{o}C to 25^{o}C
Pressure	0 to 6 bar
Density	0.00001 g.cm^{-3}
Temperature	0.01 ^{o}C
Minimum sample volume	approx. 2 ml
Dimensions (L×W×H)	400×225×231 mm
Weight	approx. 15 kg

III.2.4.4. Viscosity Measurement

Brookfield DV-III Ultra Programmable Rheometer:

The viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The viscosities were obtained using the following equation

$$\eta = (100 / RPM) \times TK \times \text{torque} \times SMC$$

Where, RPM, TK (0.09373) and SMC (0.327) stands for the speed, viscometer torque constant and spindle multiplier constant, respectively. The calibration of the instrument was done using the standard sample solutions such as distilled water and aqueous $CaCl_2$ solutions. Brookfield Digital TC-500 thermostat bath was used to maintain the temperature.

Instrument Specifications:

Speed Range	0-250 RPM, 0.1 RPM increments
Viscosity Accuracy	±1.0% of full scale range for a specific spindle running at a specific speed.
Temperature sensing range	-100°C to 300°C (-148°F to 572°F)
Temperature accuracy	±1.0°C from -100°C to 150°C ±2.0°C from +150°C to 300°C
Analog Torque Output	0 - 1 Volt DC (0 - 100% torque)
Analog Temperature Output	0 - 4 Volts DC (10mv / °C)

III.2.4.5. Refractive Index Measurement

Refractive index was be measure with the help of Digital Refractometer (Mettler Toledo 30GS) having accuracy of the instrument is ±0.0005.

Instrument Specifications:

Model	Refracto 30GS
Measurement range	1.32 -1.65
Resolution	0.0001
Accuracy	+/- 0.0005
Measurement range BRIX	0 - 85 Brix%
Resolution	0.1 Brix%
Accuracy	+/- 0.2 Brix%
Temperature range	10 - 40°
Resolution of temperature	0.1°
display	°C or °F
Trade Name	51324660

The ratio of the speed of light in a vacuum to the speed of light in another substance is defined as the index of refraction (aka refractive index or n_D) for the substance.

$$\text{Refractive index of substance } (n_D) = \frac{\text{Speed of light in vacuum}}{\text{Speed of light in substance}}$$

$$\frac{V_A}{V_B} = \frac{sin\theta_A}{cos\theta_B} = \frac{n_A}{n_B}$$

III.2.4.7. FTIR Measurement

Infrared spectra were recorded in 8300 FTIR spectrometer (Shimadzu, Japan).

III.2.4.8. UV-VIS Spectra Measurement

We used Agilent 8453 model to record the absorption pattern of UV-visible spectra.

Instrumental Specifications

OPTICAL PERFORMANCE	
Wavelength range	190-1100nm
Slit width	1nm
EP Resolution Test	> 1.6
Stray Light	< 0.03%

Wavelength Accuracy	< ±
Wavelength Reproducibility	< ± 0.5nm
Photometric Accuracy	< ± 0.005A
Photometric Noise	<0.0002A
Photometric Stability	<0.001A/h
Baseline Flatness	<0.001A
Typical Scan Time	1.5 second
Shortest Scan Time	0.1 second
Time Until Next Scan	0.1 second
PHYSICAL DIMENSIONS	
Height×Width×Depth×Weight	185×344×560nm
POWER REQUIREMENTS	
Line Voltage	90-264V AC
Line Frequency	47-63 Hz
Power Consumption	70 VA
ENVIROMENTAL CONDITIONS	
Operating Temperature	0-50^0 C
Non-Operating Temperature	40^0-70^0 C
Humidity	< 95% at 25-40^0 C

III.2.4.9. QuantaMaster 40 spectrofluorometer

Fluorescence is the emission of light from a molecule resulting from a transition from one electronic state to a lower electronic state of the same multiplicity. The most commonly observed fluorescence from organic molecules is caused by a transition from an excited singlet state to the ground singlet state.

Detection Limit	460 attomolar fluorescein in 0.1 M NaOH
Signal to Noise Ratio	10,000:1 or better (350 nm excitation, 5 nm spectral bandpass, 1 s integration time)
Data Acquisition Rate	50,000 points/sec. to 1 point/100 sec
Inputs	4 analog (+/- 10 volts)
	2 photon counting (TTL)
	1 analog reference channel (+/- 10 volts)
	2 TTL
Outputs	2 analog (+/- 10 volts)
	2 TTL
Emission Range	185 nm to 680 nm (optional to 900 nm)

Light Source	High efficiency continuous Xenon arc lamp
Monochromators	Czerny-Turner design
Focal Length	200 mm
Excitation Grating	1200 line/mm 300 nm blaze
Emission Grating	1200 line/mm 400 nm blaze
Optional Grating	75 to 2400 line/mm and holographic models available
Bandpass	0 to 24 nm, continuously adjustable. (computer control available)
Wavelength Accuracy	+/- 0.5 nm
Wavelength Resolution	0.06 nm
Detection	Photon counting/analog
System Control	Computer interface with spectroscopy software
Dimensions	38 x 30 inches

The wavelengths of fluorescent light emitted by a sample are measured using a monochromator, holding the excitation light at a constant wavelength. This is called an *emission spectrum*. An **emission map** is measured by recording the emission spectra resulting from a range of excitation wavelengths and combining them all together. This is a three dimensional surface data set: emission intensity as a function of excitation and emission wavelengths, and is typically depicted as a contour map.

III.2.4.9. NMR Spectroscopy

Most of the NMR spectra were recorded in D_2O(δ 4.79 ppm). We also used d6-DMSO (internal standard TMS, δ 2.50 ppm) as NMR solvent. ^{1}H NMR spectra were recorded using 400 MHz and 600 MHz BRUKER AVANCE.

III.2.4.10. HRMS Measurement

HRMS was done using Agilent Accurate-Mass Q-TOFLC/MS6520.

III.2.4.11 Scanning Electron Microscope

Scanning Electron Micrographs were recorded using JEOL JSM-1100 instrument.