Characterization

3.1 Thin Layer Chromatography(TLC)

Prior to E. Stahl discovery of an easy way to create uniform layers, combining the numerous parts of technology into a single fundamental entity, standardising the chromatographic conditions and the absorbents, as well as researching different kinds of compounds, thin layer chromatography (TLC) was largely dismissed [1]. The amount of time it takes is negligible and the necessary equipment for thin-layer chromatography is straightforward and affordable. TLC theory and application are covered in-depth by I. M. Hais, G. B. Bettol-Marini, K. Macek and E. Stahl, B. Fried and J. Sherma, and G. Kirchner [2-6].

TLC is usually more effective than paper chromatography at separating reactive dyes [7]. Under multiple chromatographic separation conditions, A. Cee and J. Gasparic used TLC to carefully evaluate vinyl sulphone reactive dyes of the Remazol class. Due to the lack of substantivity to cellulose, resolution is improved and the dyes do not stay at the beginning position [8]. J. Parkvee and M. Perpar developed the silica gel-G layers with the solvents after applying 0.1% aqueous solution of reactive dyes [9].

(I) Ethyl acetate-n-Propanol-water (10:60:30), advisable for separating Levafix dyes from Bayer, Primazine dyes from BASF, Reactive dyes from Geigy, Remazol dyes from Hoechst, and Drimaren dyes from Sandoz.

(II) Pyridine-2-Butanol-ammonium hydroxide-ethanol-2-Butanol (20:40:30:10) able to distinguish between monoazo and bisazo reactive dyes.

The following conditions were used for the thin layer chromatography of the synthesized reactive dyes:

Solvent Composition: DMF + Benzyl alcohol + Water (2:3:3)

The spotting solvent: DMF

Temperature: 31-32ºC

Six hours for chamber saturation. The findings are shown in Tables:2,3,4,5,6 &7.

3.2 Ultraviolet (UV) and Visible Spectroscopy

Ultra violet as well as Visible spectroscopy seems to be the study of how electromagnetic waves in the UV and Visible frequency categories of electromagnetic interference are absorbed. The process of absorption UV-vis spectroscopy is one of the best

tools for evaluating dye aggregation characteristics as a function of concentration. There have been several UV-Vis spectroscopy research on aggregation, but their results are sometimes inconsistent and in some cases, conflicting between 10 and 400 nm UV spectrum. It is divided into two regions: far ultraviolet (vacuum) (10-200 nm) and near ultraviolet (quartz) (200-400 nm). The visible spectrum ranges approximate frequency between 400 to 800 nm.

An electron gets excited from a lower to higher molecular orbital as a result of the absorption of electromagnetic radiation in the visible and ultraviolet ranges (electron energy level). Because electronic transitions are involved in UV and visible spectroscopy, the term "spectroscopy" is commonly applied. The primary purposes of ultraviolet and visible spectroscopy for organic chemists are to identify conjugated multiple bonds and aromatic rings and to determine whether they are present.

A UV-Visible spectrophotometer, in accordance with the Lambert-Beer law, captures the visible or ultraviolet (UV) frequency band represents a plot point of frequencies of absorbed radiations vs. the strength of absorption represented in terms of absorbance (optical density) A or quantitative absorption coefficient (molar extinction coefficient).

$$A = \log_{10}\left(\frac{I_o}{I}\right) = \epsilon l c$$

Wherein Io is the radiation frequency at impact and I is indeed the radiation intensity following passing as a result of test solution, A represents the optical density absorption, ε the molar absorbed coefficient or molar elimination coefficient, c is indicating concentrations of the chemical (mole/litre) and l represent the length of the sample path (cm). At a particular wavelength, the molar amount of light absorbed of an organic substance is constant. At maximum absorption, the molar absorptivity is max or log10max, is typically used to express the strength of a wavelength within the ultraviolet or visible spectrum absorption peak. The symbol for the maximum absorption's wavelength is max. All ʎmax values of dyes are recorded in Tables:7 and Figure:9,10 &11 display a few selected spectral curves.

3.3 IR Spectra

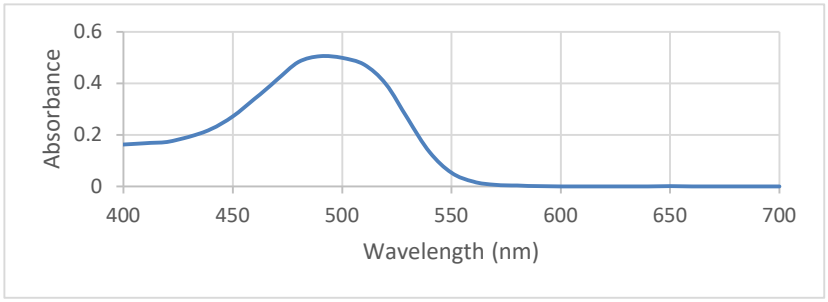
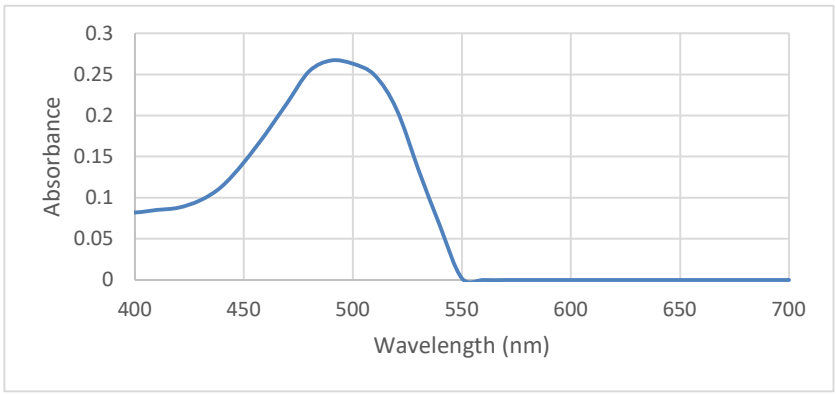
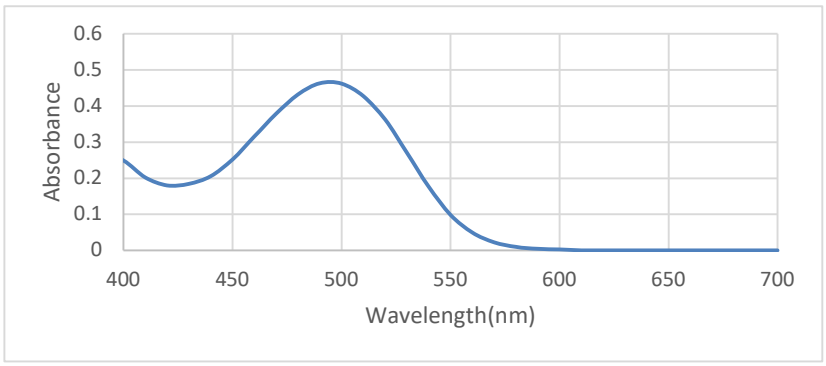
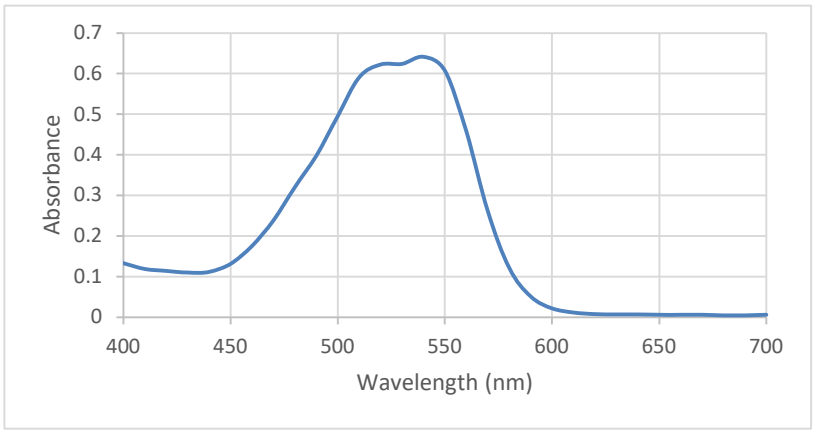
To identify and qualitatively characterise structural components of unidentified substances, infrared spectroscopy is frequently used in organic chemistry. IR is also effective for elucidating and validating the structures of known and new natural chemical substances. In the dye and dye intermediates industries, infrared spectroscopy is commonly utilized. The Majority of spectrometers used in industry serve this purpose, however theoretical physicists can also use the spectra to gather essential information about the physics of small molecules. The earlier investigations are particularly beneficial to the analyst since they pinpoint the precise movements associated with the many distinct frequencies, permitting the analyst to, in some degree, evaluate the possibility of frequency shifts happening the modifications in the group's atmosphere.

For the purpose of characterising the dyes, the IR spectral properties are used. In actuality, it is a very effective approach for identifying textiles colorant, containing traces of unreacted intermediates or isomer forms. This technique is frequently used to check for simultaneity in dye production. The compounds IR spectra may, however, represent unique characteristics such as cis-trans isomerism, which refers to the relative position of coupling when many coupling sites are accessible. The preferred spectral curves are displayed in Figure:8,9&10.

The entire molecule generates an infrared spectrum, but specific absorption bands are formed by common or distinctive atom groups, like double-bonded carbon, the >C=O group, or the -OH group. With ketone, ester, and conjugated carbonyl complexes, the absorption band for the carbonyl group is at a different frequency because the group frequency around the functional group is environmentally reactive. By utilising IR, chemists can use these vibrations of absorption to assign structure to organic molecules. Between 4000 cm^{-1} and 450 cm^{-1} are the frequencies that can be used to practically identify group existence.

The range of wavelengths in the infrared spectrum between 1400 cm^{-1} and 600 cm^{-1} is known as the fingerprint region. The number of bands and their relative intensities are typical, even though both bands may not be assigned here.

Figure:9 UV-Visible Spectroscopy of Dyes

Z1	 Absorbance 0.6 0.4 0.2 0 400 450 500 550 600 650 700 Wavelength (nm)
Z4	 Absorbance 0.3 0.25 0.2 0.15 0.1 0.05 0 400 450 500 550 600 650 700 Wavelength (nm)
Z10	 Absorbance 0.6 0.5 0.4 0.3 0.2 0.1 0 400 450 500 550 600 650 700 Wavelength(nm)
Z13	 Absorbance 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0 400 450 500 550 600 650 700 Wavelength (nm)

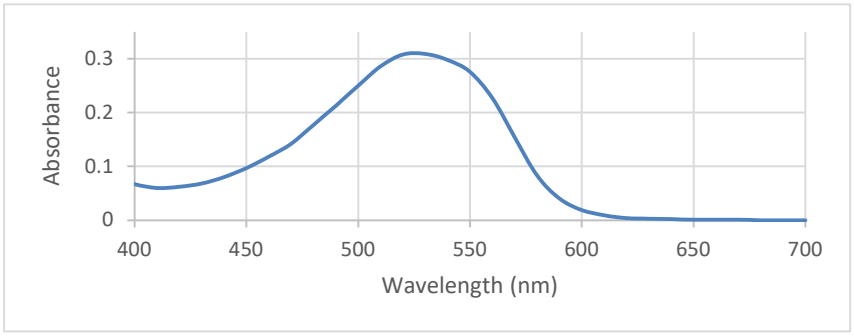
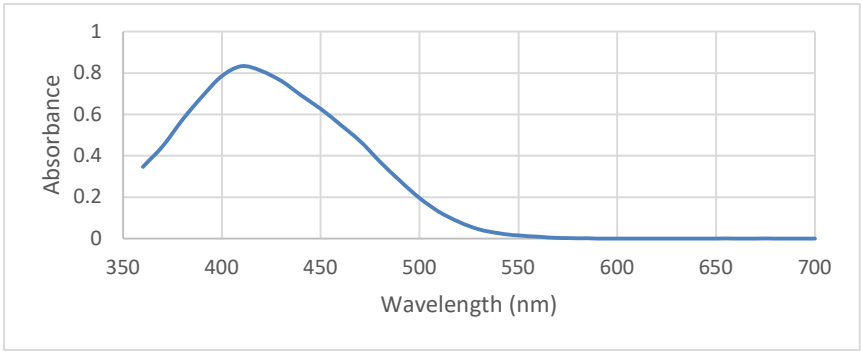
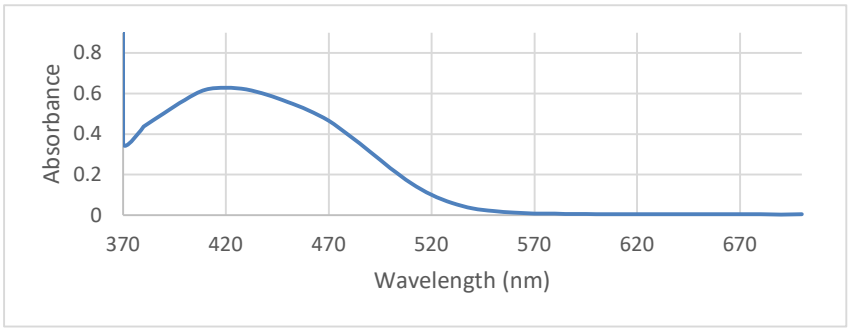
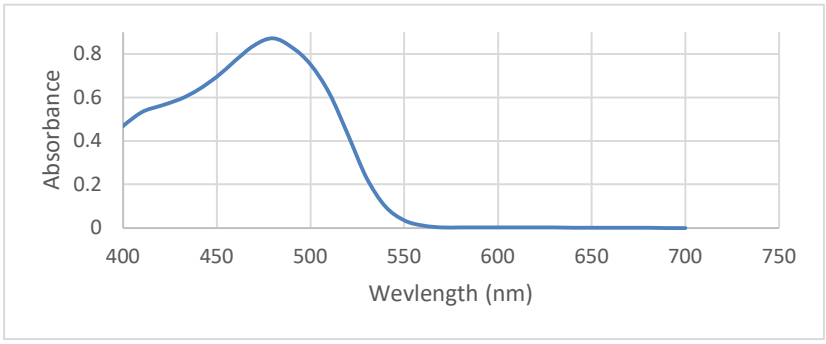
Z20	
Z22	
Z28	
Z34	

Figure:10 UV-Visible Spectroscopy of Dyes

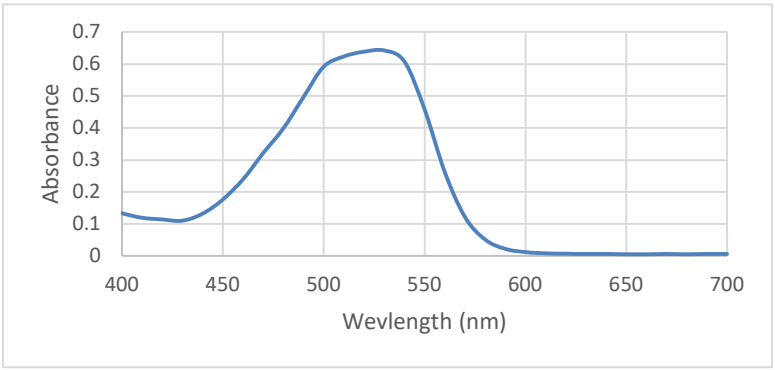
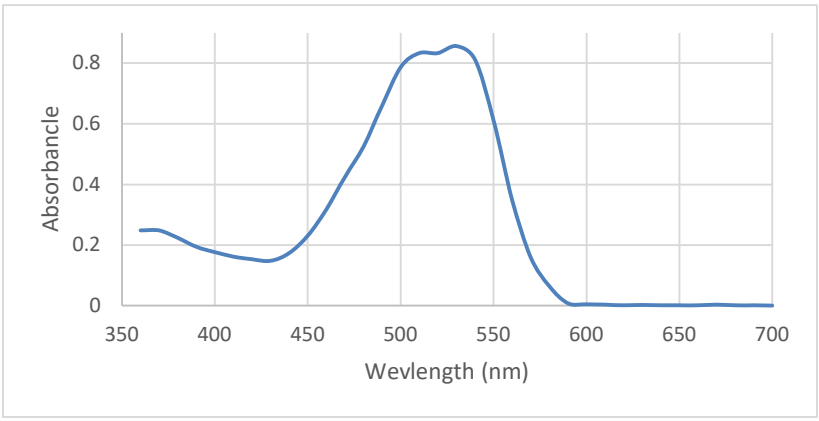
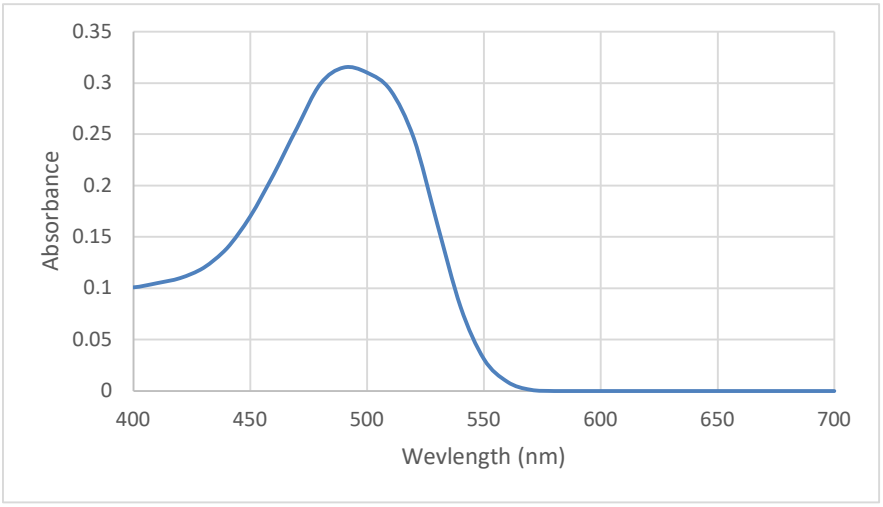
Z43	
Z48	
Z54	

Figure:11 UV-Visible Spectroscopy of Dyes

Dye No.	λ_{max}	Log ε	Dye No.	λ_{max}	Log ε	Dye No.	λ_{max}	Log ε
Z1	490	4.46	**Z19**	432	4.33	**Z37**	509	4.15
Z2	450	4.41	**Z20**	530	5.08	**Z38**	488	4.37
Z3	480	4.43	**Z21**	576	5.93	**Z39**	465	4.3
Z4	510	5.04	**Z22**	470	4.66	**Z40**	431	4.29
Z5	513	5.14	**Z23**	489	4.73	**Z41**	580	5.29
Z6	520	5.12	**Z24**	476	4.85	**Z42**	506	4.17
Z7	460	4.36	**Z25**	511	4.91	**Z43**	544	5.74
Z8	512	5.45	**Z26**	518	4.76	**Z44**	503	4.09
Z9	523	5.39	**Z27**	526	4.6	**Z45**	434	4.35
Z10	500	5.18	**Z28**	450	4.1	**Z46**	451	4.25
Z11	522	5.2	**Z29**	509	4.09	**Z47**	467	4.1
Z12	535	5.81	**Z30**	519	5.11	**Z48**	550	4.87
Z13	540	5.67	**Z31**	537	5.34	**Z49**	485	4.23
Z14	534	5.32	**Z32**	545	5.13	**Z50**	496	4.55
Z15	510	4.92	**Z33**	533	5.19	**Z51**	590	5.67
Z16	447	4.44	**Z34**	490	4.75	**Z52**	551	5.27
Z17	487	4.77	**Z35**	501	4.28	**Z53**	571	5.1
Z18	543	5.38	**Z36**	514	4.37	**Z54**	500	4.08

Table:8 UV-Visible data of reactive dyes Z1-Z54

UV Visible spectra shows in Table:8 measured in water at 28 °C with a dye concentration of 4×10^{-6} M.

3.3.1 Characteristics of IR Spectra:

IR spectrum of synthesized dyes was carried out on Perkin-Elmer Spectro 400 IR spectrophotometer using KBr pellets at Ribosome Research Centre Pvt. Ltd., Kim.

Number of IR spectrums are shown as below:

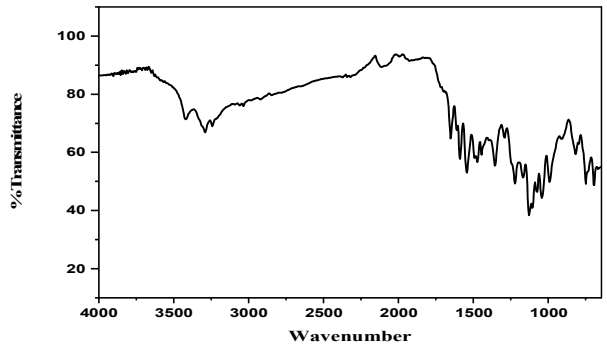
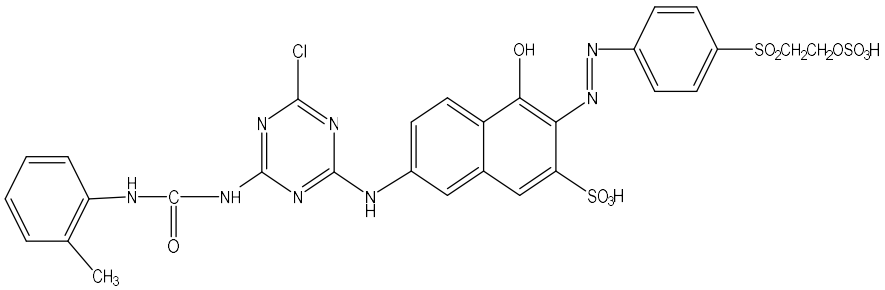


Figure:12 IR spectra of reactive dye Z2

IR in cm^{-1}: 3300-3400(O-H Stretching, functional group -OH), 3250-3295 (N-H Stretching, functional group -NH-), 1520-1640(C-N Stretching, s-Triazin ring), 1530 (-N=N- stretching), 1180-1050(S=O Stretching, functional group $-SO_3H$).



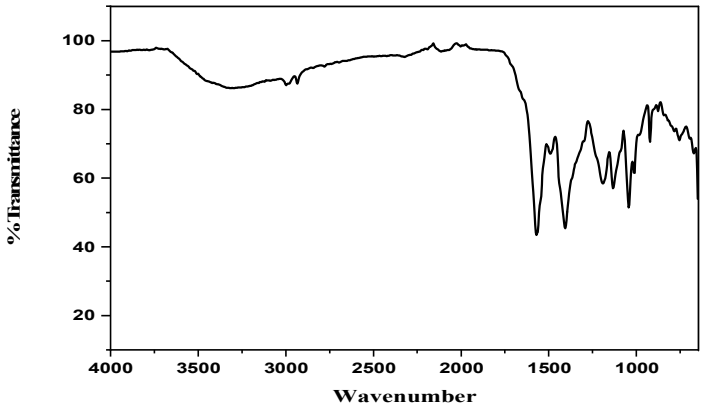
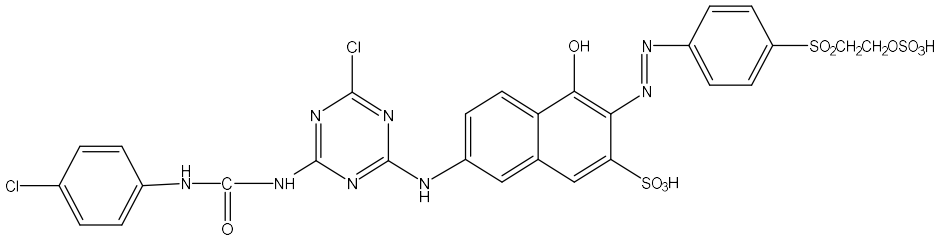


Figure:13 IR spectra of reactive dye Z9

IR in cm^{-1}: 3300-3400 (O-H Stretching, functional group -OH), 3250-3295 (N-H Stretching, functional group -NH-), 1520-1640(C-N Stretching, s-Triazin ring), 1534 (-N=N- stretching),1180-1050(S=O Stretching, functional group $-SO_3H$).



Reactive dye Z9

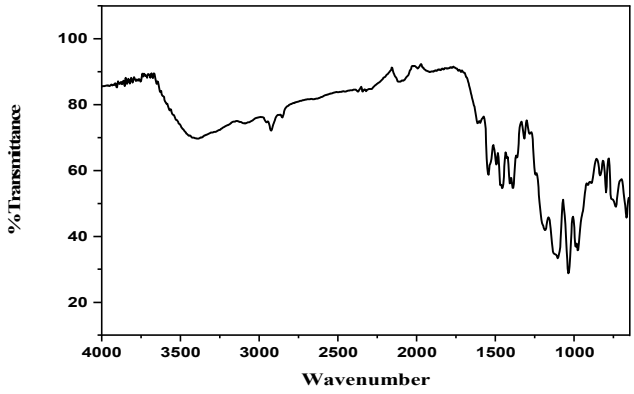
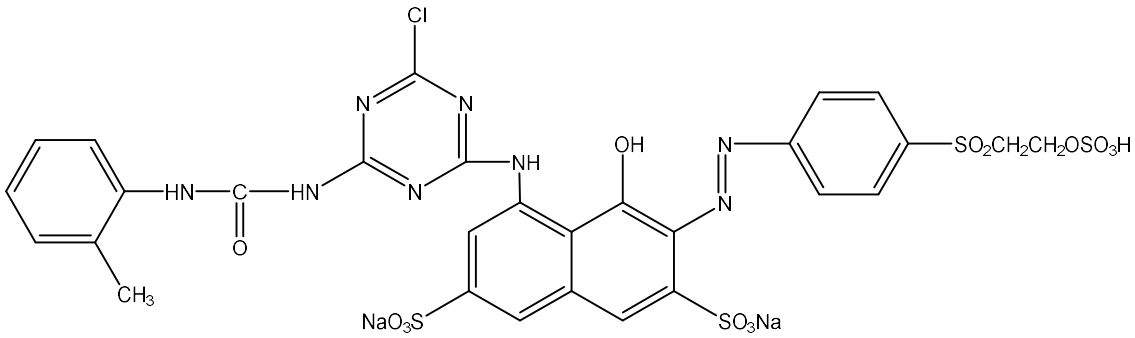


Figure:14 IR spectra of reactive dye Z12

IR in cm^{-1}: 3300 (Strong, Broad, -OH), 3200 (Strong, two bands, $-NH_2$), 3100 (Aromatic –CH Stretching), 2530 (-SH stretching), 1605 (-C=N stretching), 1531 (-N=N- stretching), 1180 & 1112 (Strong, two bands, $-SO_3H$).



Reactive dye Z12

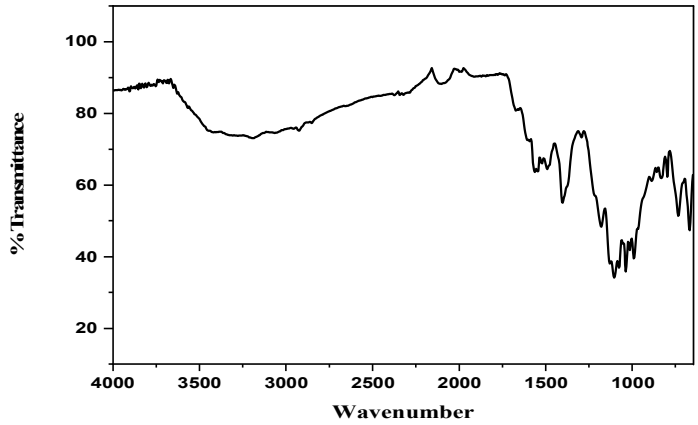
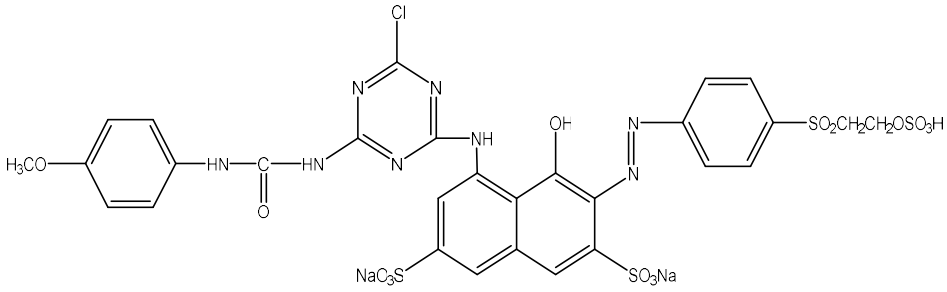


Figure:15 IR spectra of reactive dye Z20

IR in cm^{-1}: 3350 (Strong, Broad, -OH), 3250 (Strong, two bands, $-NH_2$), 3150 (Aromatic –CH Stretching), 2520 (-SH stretching), 1610 (-C=N stretching), 1527 (-N=N- stretching), 1185 & 1110 (Strong, two bands, $-SO_3H$).



Reactive dye Z20

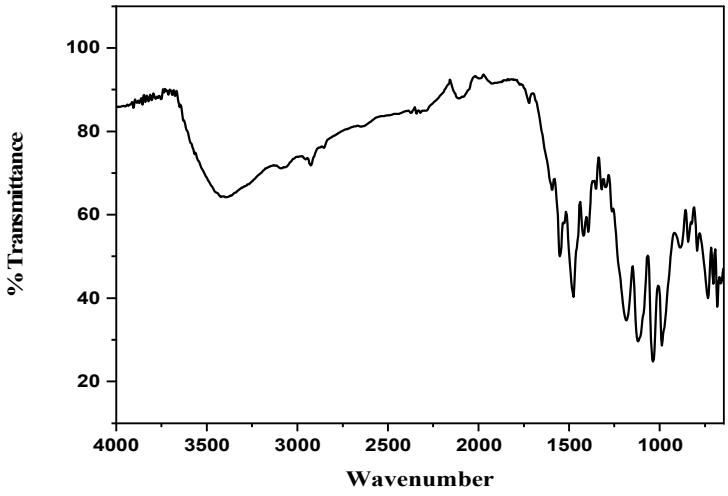
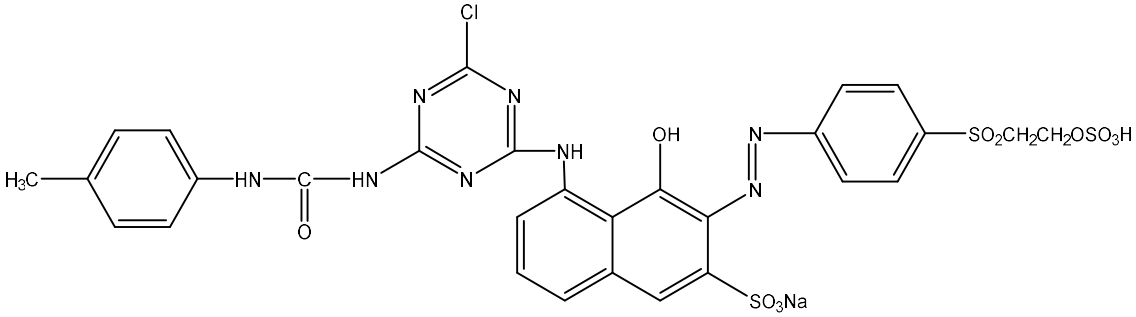


Figure:16 IR spectra of reactive dye Z24

IR in cm^{-1}: 3420 (Strong, Broad, -OH), 3255 (Strong, two bands, $-NH_2$), 3100 (Aromatic –CH Stretching), 2515 (-SH stretching), 1615 (-C=N stretching), 1532 (-N=N- stretching), 1180 & 1115 (Strong, two bands, $-SO_3H$),



Reactive dye Z24

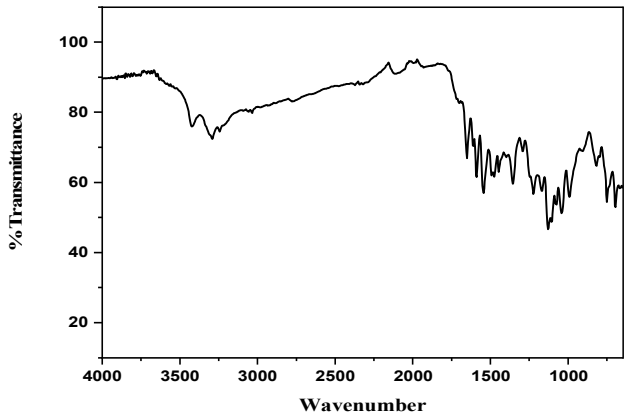
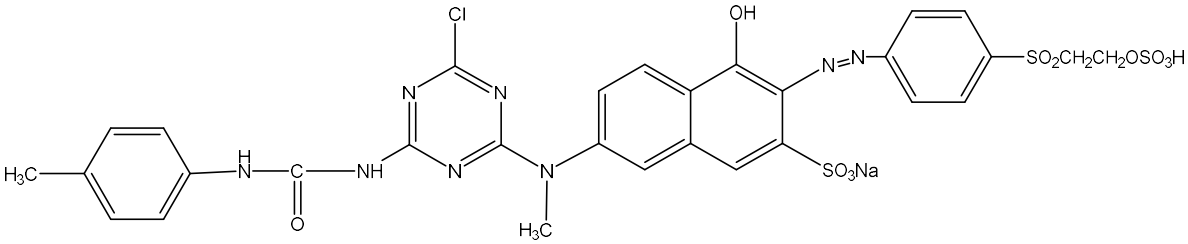


Figure 17: IR spectra of reactive dye Z33

IR in cm^{-1}: 3400 (Strong, Broad, -OH), 3250 (Strong, two bands, $-NH_2$), 3000 (Aromatic –CH Stretching), 2525 (-SH stretching), 1625 (-C=N stretching), 1537 (-N=N- stretching), 1185 & 1125 (Strong, two bands, $-SO_3H$).



Reactive dye Z33

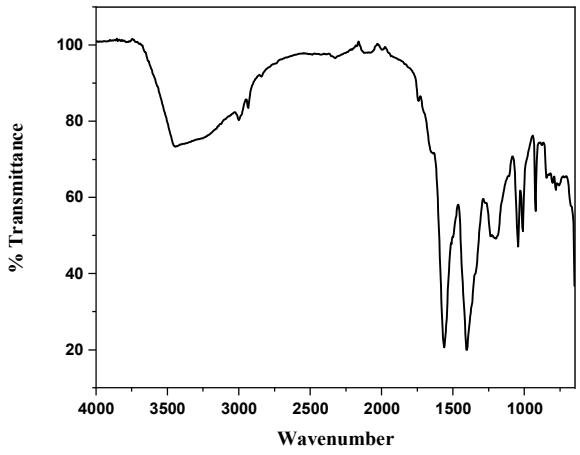
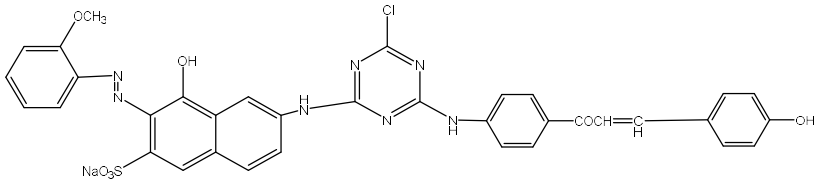


Figure:18 IR spectra of reactive dye Z49

IR in cm^{-1}: 3500 (Strong, Broad, -OH), 3200 (Strong, two bands, $-NH_2$), 3010 (Aromatic –CH Stretching), 2520 (-SH stretching), 1610 (-C=N stretching), 1537 (-N=N- stretching), 1180 & 1130 (Strong, two bands, $-SO_3H$).



Reactive dye 49

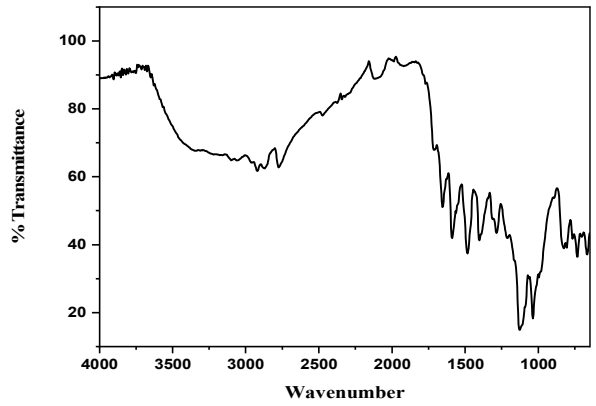
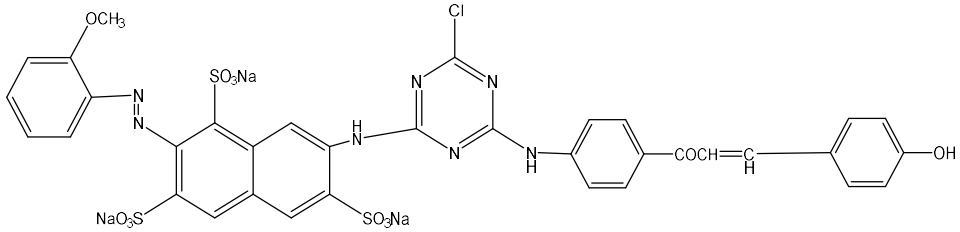


Figure:19 IR spectra of reactive dye Z53

IR in cm^{-1}: 3400 (Strong, Broad, -OH), 3200 & 3300 (Strong, two bands, -NH_2), 3050 (Aromatic –CH Stretching), 2510 (-SH stretching), 1606 (-C=N stretching), 1531 (-N=N- stretching), 1185 & 1120 (Strong, two bands, -SO_3H).



Reactive dye Z53

3.4 Nuclear Magnetic Resonance Spectroscopy

Comparable to infrared or ultraviolet spectroscopy, NMR spectroscopy is just another type of absorption spectroscopy that is extremely useful for determining the structures of chemical compounds [16-18]. NMR phenomenon was first experimentally discovered in 1945, while becoming theoretically promised considerably earlier [19]. A molecule has the ability to absorb electromagnetic radiation at frequencies determined by the molecule's characteristics in the radiofrequency area when the conditions are right. NMR spectrum includes of absorption as a function of individual peaks against peak strengths.

Until after the 1951 discovery of three different signals in the spectrum of ethyl alcohol. This is in accordance with the vibration of that same protons of methyl, methylene and hydroxy made it viable to use NMR spectroscopy to examine the structure of complex

molecules[20] and that additional fine splitting that is dependent on the amount and kind of magnetic nucleus present in the molecule is caused by magnetic nuclei in different groups that are present in liquid molecules are communicating[21]. To distinguish it from spectra including protons such as ^{13}C, ^{14}N or ^{19}F the spectrum is typically referred to as a proton magnetic resonance[PMR] spectrum. A proton was indeed the nucleus involved in this case for ethyl alcohol.

3.4.1 Chemical shift:

In the presence of an intense magnetic field, an unpaired atom Ho, according to electromagnetic field laws, the electrons have a field direction that is opposite to that of the nucleus (H'), which reduces the amount of external field that would have been there had the electron shell not been present. As a result, it takes a somewhat stronger external field to create resonance of diamagnetically screened nuclei and the strength of this increase varies depending on the electronic surroundings of the nucleus. Nuclear resonance signal shift brought on by electrical surroundings. It's referred to as chemical shift. The measurement that simplifies the procedure to express a chemical shift is parts per million (ppm) of the total magnetospheres.

3.4.2 Multiplicity Assessment Procedures:

Many basic standards to assess the multiplicity of such a signal have been constructed, although they typically only apply when AV / J > 6. (In which AV in H2 reflects the separating of the frequencies of the interacting groups). When connected, comparable protons do not split apart.

- The dimensions (i.e., J values) within this kind of multiplier are identical to the band gaps produced through mutual coupling.
- The multiplicity of an equivalent proton group is (n + 1), in which n is indicate the number of same protons inside the functional group that's also associated to the initial group.
- A cluster of comparable protons seems to have a multiplicity of equal to $(n_a + 1)(n_b + 1)$ when it is connected to another a collection of protons that are identical, in which na symbolizes one group and nb indicates another, etc.

3.4.3 NMR spectra Characteristics:

At Central Instrumental Facility, Savitribai Phule University Pune. The NMR spectra of dyes were carried out in DMSO and shown as below:

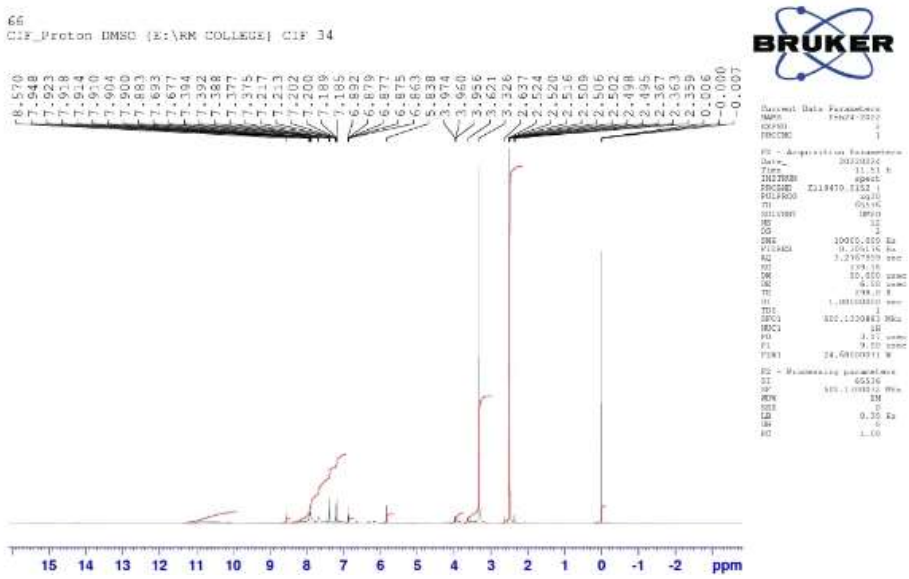
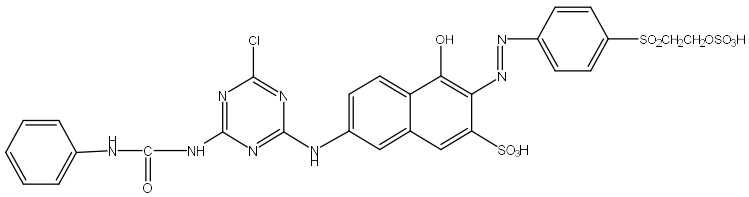


Figure: 20^{1}H NMR Spectrum of reactive dye Z11



Reactive dye Z11

^{1}H-NMR (DMSO, δ ppm): 10.45 (1H, s, -CONH-), 10.10 (2H, s, Ar-NH-), 8.57, 8.50 (3H, s, -OH), 7.90-7.07 (13H, m, Ar-H), 4.07 (2H, t, -OCH_2-), 3.60 (2H, t, -SO_2CH_2).

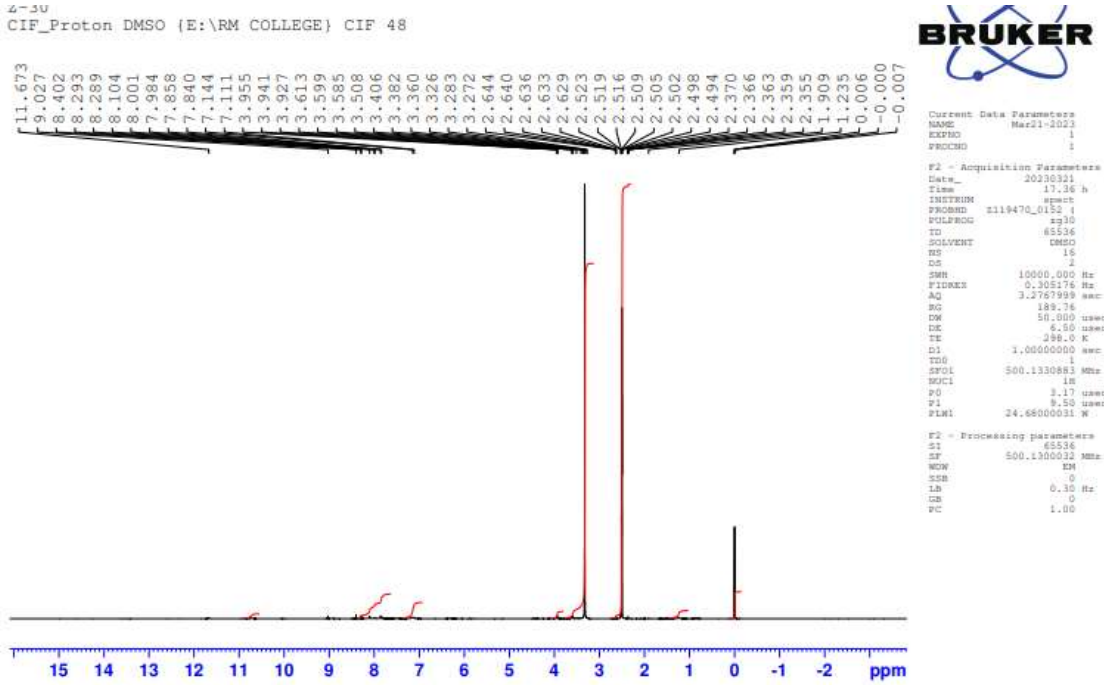
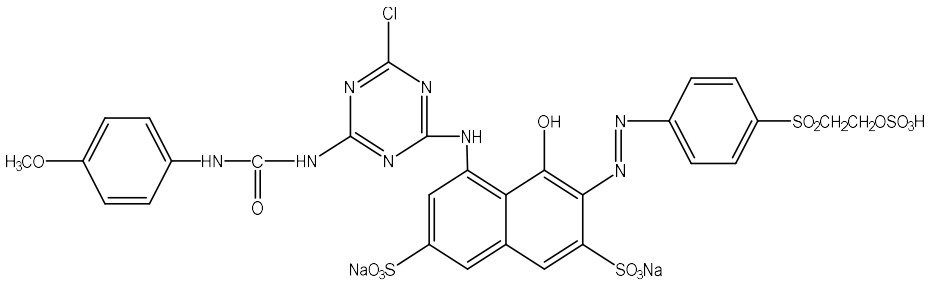


Figure:21 [1]H NMR Spectrum of reactive dye Z20



Reactive dyeZ20

[1]H-NMR (DMSO, δ ppm): 10.50 (1H, s, -CONH-), 10.01 (2H, s, Ar-NH-), 9.01, 8.50 (2H, s, -OH), 8.29-7.00 (11H, m, Ar-H), 4.09 (2H, t, $-OCH_2-$), 3.90 (3H, s, $-OCH_3$), 3.60 (2H, t, $-SO_2CH_2-$).

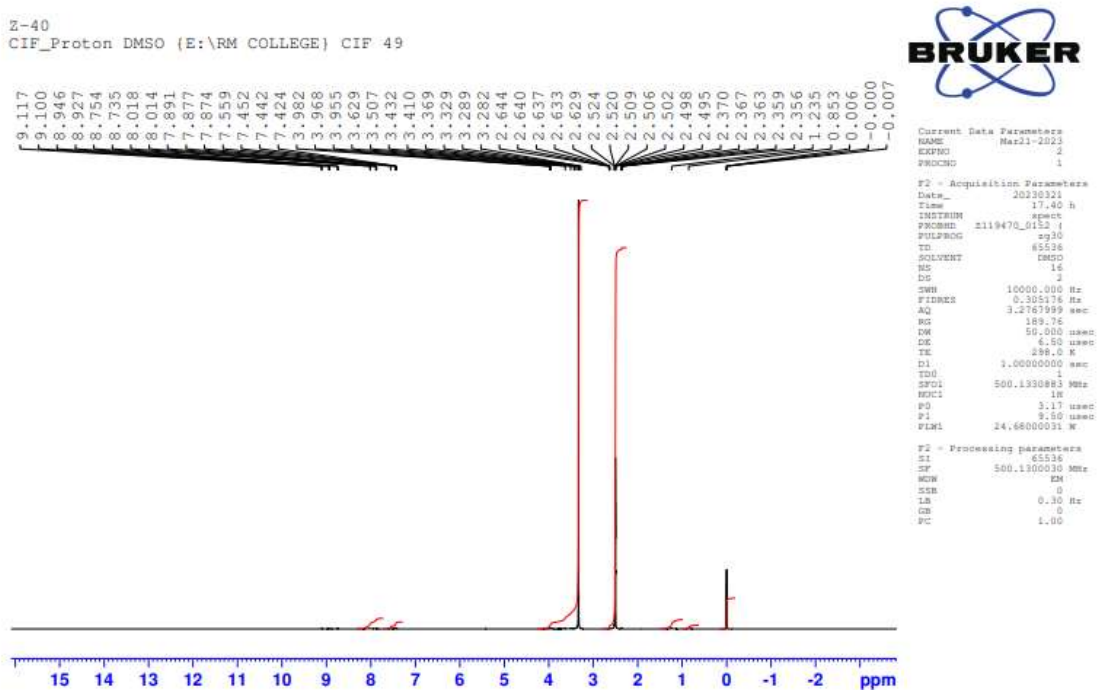
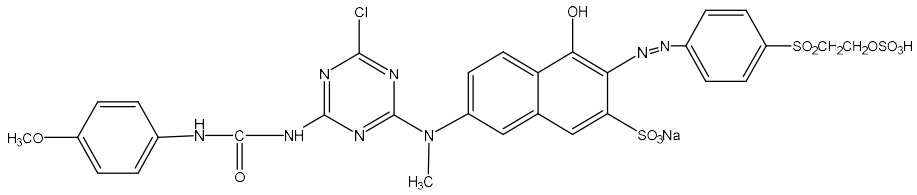


Figure:22 ^{1}H NMR Spectrum of reactive dye Z40



Reactive dye Z40

^{1}H-NMR (DMSO, δ ppm): 9.11 (1H, s, -NH-), 9.10 (1H, s, -NH-CO-NH-), 8.75 (1H, s, -SO_3H), 8.73 (1H, s, -OH), 8.01-7.42 (9H, m, Ar-H), 3.98 (2H, t, -OCH_2-), 3.95 (3H, s, Ar-O-CH_3), 3.62 (2H, t, -CH_2-CH_2-), 3.32 (1H, t, Ar-H), 3.28 (3H, s, N-CH_3), 2.50, 2.35 (2H, d, Ar-H).

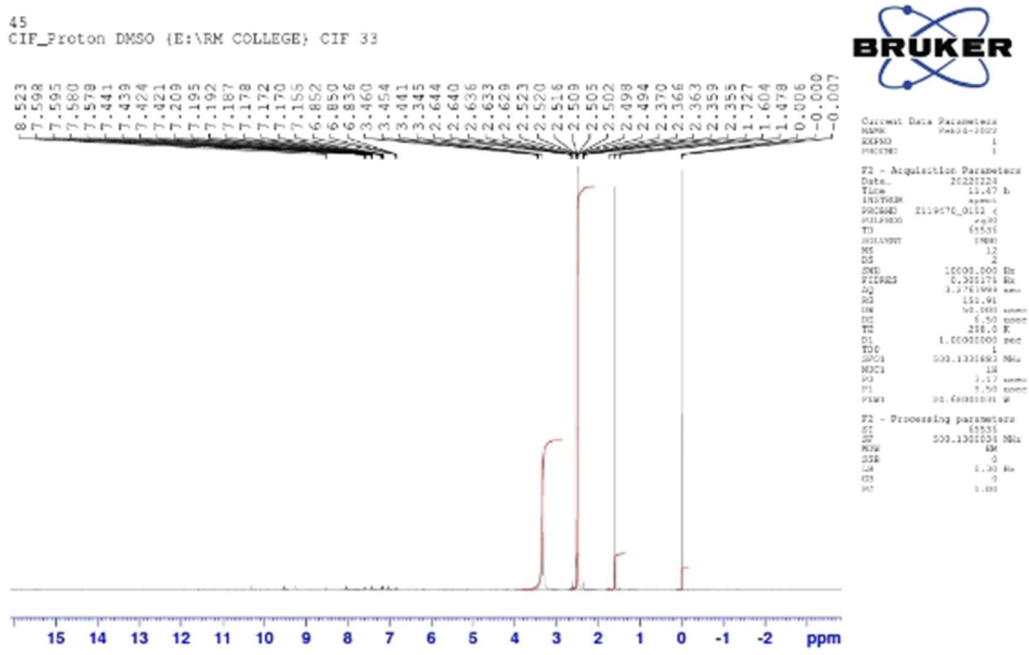
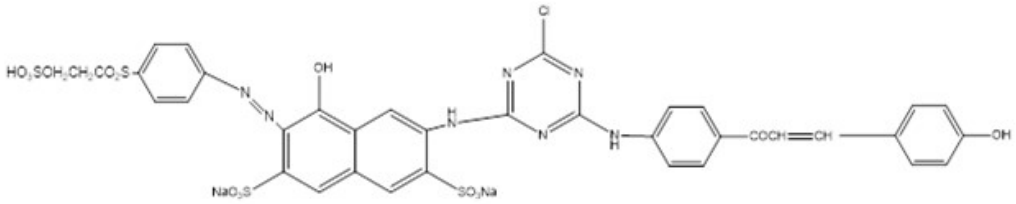


Figure:23 [1]H NMR Spectrum of reactive dye Z41



Reactive dye Z41

[1]H-NMR (DMSO, δ ppm): 10.48, 8.36 (2H, s, Ar-NH-), 9.68 (1H, s, -OH), 8.52 (2H, s, -OH), 8.16 (1H, d, Ar-CH_2-), 7.60 (1H, d, -$COCH_2$-), 7.59-7.10(18H, m, Ar-H), 3.46 (2H, t, -SO_2CH_2), 3.44 (2H, t, -CH_2-SO_3H).

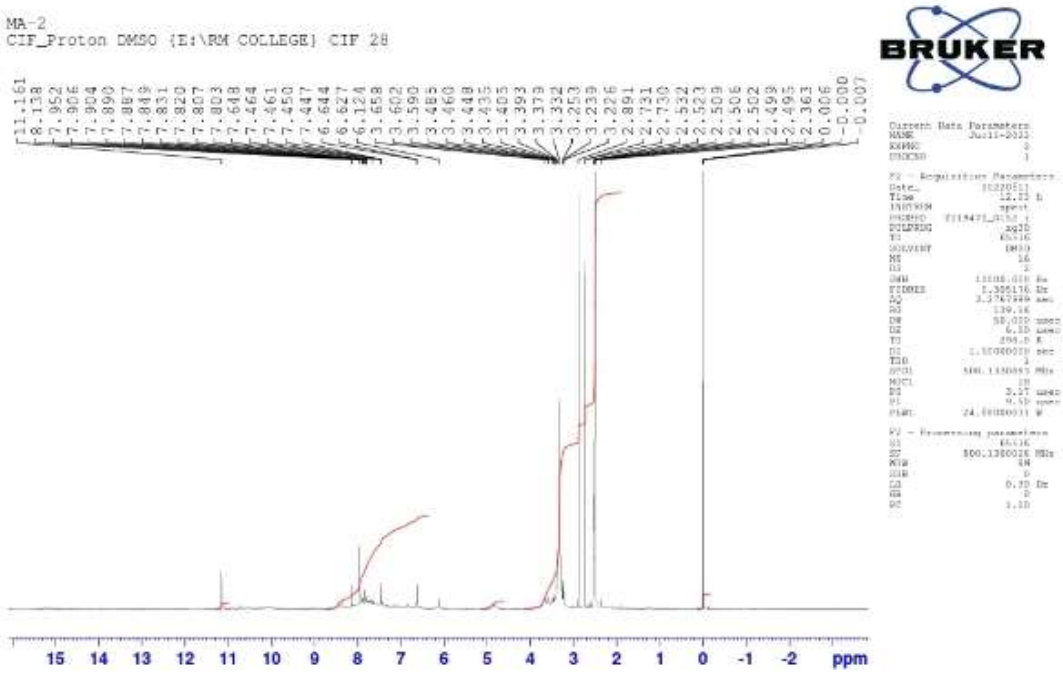
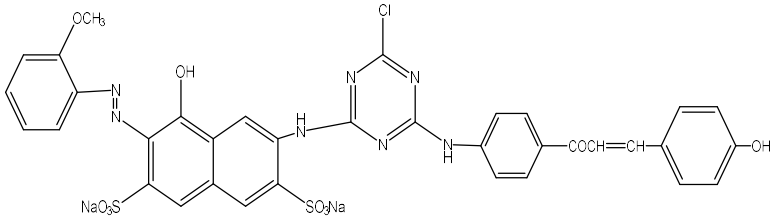


Figure:24 ^{1}H NMR Spectrum of reactive dye Z48



Reactive dye Z48

^{1}H-NMR (DMSO, δ ppm): 11.16 (2H, s, Ar-NH-), 8.50 (2H, s, -OH), 8.13 (1H, d, Ar-CH_2-), 8.10-7.43 (15H, m, Ar-H), 7.50 (1H, d, -COCH-), 3.65 (3H, s, -OCH_3).

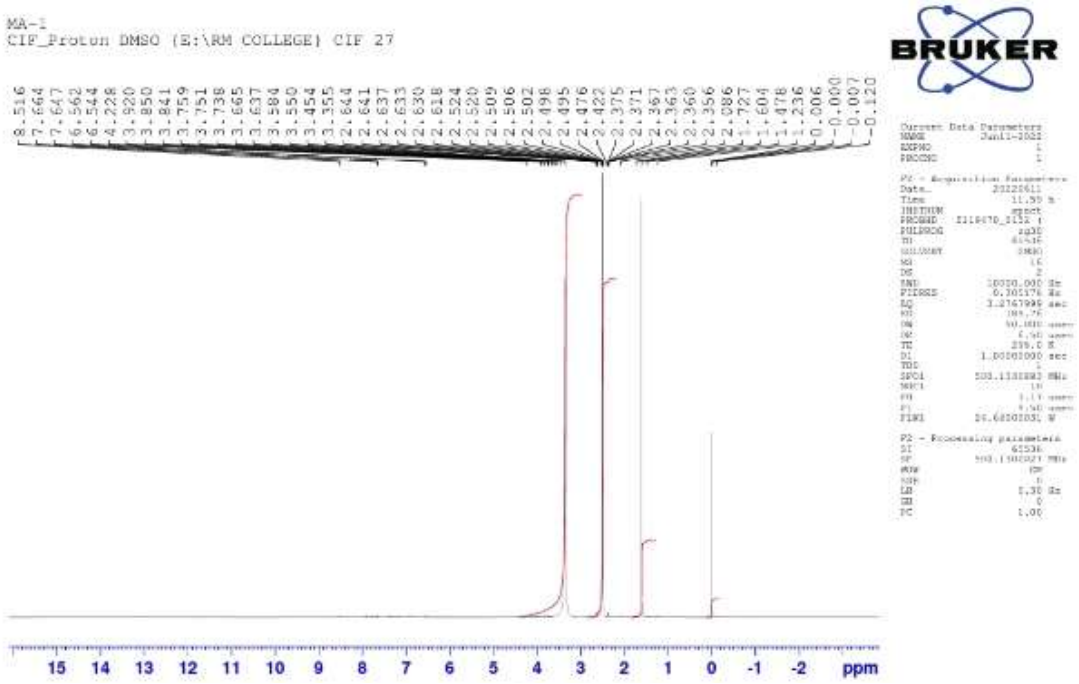
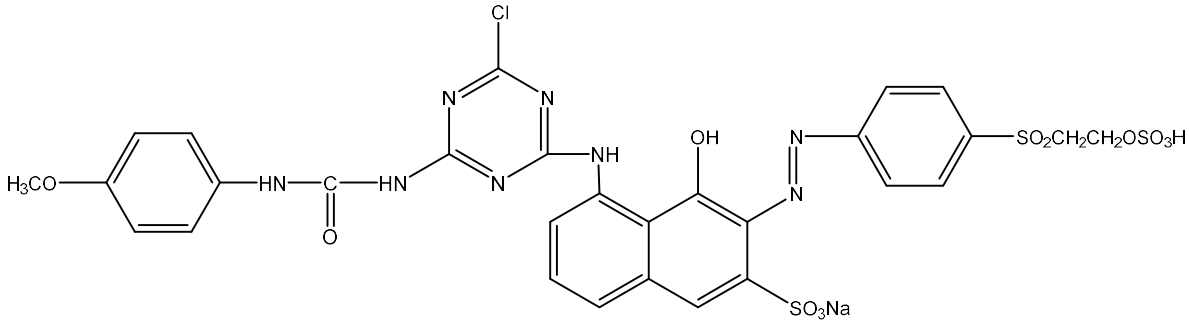


Figure:25 [1]H NMR Spectrum of reactive dye Z30



Reactive dye Z30

[1]H-NMR (DMSO, δ ppm): 9.02 (2H, s, -NH-CO-NH-), 9.0 (1H, s, -OH), 8.4 (1H, s, Ar-NH-), 8.29 (1H, s, -OH), 8.40-7.11 (12H, m, Ar-H), 3.95 (2H, t, -CH_2-CH_2-), 3.92 (3H, s, Ar-OCH_3), 3.61 (2H, t, -CH_2-CH_2-).

3.5 References

1. E Stahal, *Chemiker Ztg,* (1958), **82,** 323.
2. E Stahl Thin-Layer Chromatography A Laboratory Hand Book 2nd Ed.,*Springer Academic Press,*(1969).

3. B Fried and J Sherma, Thin Layer Chromatography Techniques and Application, *Marcel Dekker,* (1982), **55,** 71-85.
4. J G Kirchner, *Journal of chomatrography,* (1979), **17**, 201-208.
5. B Bettolo-Marini, Thin Layer Chromatography, *Elsevier,* (1964), 525.
6. K Macek and IM Hais, Stationary Phase in Paper and Thin Layer Chromatography,*Elsevier,* (1965).
7. J Reif Dtsch, *Textiles -Tech,* (1963), **13**, 86.
8. A Cee and J Gasparic, *Collect.Czech. commu.,* (1968), **83**, 2406-2421.
9. J Parkvee, M Perpar, *Anal.Chemistry,* (1965), **206**, 356.
10. D M Lewis, *Colouration technology*, (2014), **130**, 318-412.
11. L M Jackman, S Stemhell, Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, *Pergamon*, Oxford,2nd edition,(1969).
12. F A Bovey,Nuclear Magnetic Resonance Spectroscopy, *Academic Press*, New York (1969).
13. F C Nachold and J J Zuckerman, Determination of Organic Structures by Physical Methods, *Academic Press*, New York (1971), **4**.
14. C I Grotter, Phisica, (1936), **3**, 995.
15. T Arnold, S S. Dharmati and M. E. Packard, *J. Chem. Phys*., (1951), **19**, 507.
16. H S Gutowsky, D W Mc Call, C P Slichter, *Phys. Rev*., (1951), **84**, 589.