WWJMRD 2015; 1(5): 48-52
www.wwjmrd.com
International Journal
Peer Reviewed Journal
Refereed Journal
Indexed Journal
E-ISSN: 2454-6615

Braham Dutt Arya
Department of Chemistry,
Pt. Neki Ram Sharma
Government College, MDU
Rohtak, India

Neetu Malik
Department of Chemistry,
Pt. Neki Ram Sharma
Government College, MDU
Rohtak, India

Correspondence:
Braham Dutt Arya
Department of Chemistry,
Pt. Neki Ram Sharma
Government College, MDU
Rohtak, India

FTIR Spectroscopic Analysis of Various Pharmatheutically Important Organic Dyes

Braham Dutt Arya, Neetu Malik

Abstract
Organic dyes have emerges as an effective candidate for a number of biological applications. These dyes finds applications in drug delivery, cancer therapy, photodynamic and photothermal therapy and for various biological staining purposes. Hence, for effective attachment, adsorption or binding of these dyes onto the surface of desired recipient their functional group investigation is critically required. In the present study, we investigated the functional groups present on various organic dyes of biological importance. We have reported the FTIR spectroscopic analysis of these dyes with critical and detailed functional group determination. This study will provides the basis for further application of these dyes by chemical modifications and surface- structural design with other nanosystems.

Keywords: FTIR, Rodamine B, auramine O, Methlyne blue and nile blue sulfate.

Introduction

Ever since pre-historic time, man has been fascinated to color the objects of daily use employing inorganic salts or natural pigments of vegetable, animal, and mineral origins. Dyes are colored substances which are soluble or go into solution during the application process and impart color by selective absorption of light [1]. Dyes are of two types, i.e. synthetic and natural. Synthetic dyes are based on petroleum compound, whereas natural dyes are obtained from plant, animal, and mineral matters [2]. Generally, dyes conventionally refer to organic molecule dissolved as molecular chromophores. From day to day life we have been using a lot of dyes for our day to day needs. Besides the coloring benefits of these specific organic moties they also finds numerous applications in a number of biological as well as chemical applications. For an instance, methylene blue is used as a photosensitizer by sensitizing it to specific wavelength and finds applications in photodynamic cancer treatment therapy. A number of organic dyes has been used for various biological samples staining. Today era is the era of the early diagnosis of different diseases and various organic dyes used for staining provides a versatile method for early diagnosis. Synthetic dyes has been further classified as basic or charged dyes, acidic dyes, neutral dyes, Sulphur dyes, Azoic dyes and premetalised dyes [3]. The color of the dyes as well as their electronic behavior depends upon the absorption of electromagnetic radiations in the UV and visible regions. The covalently unsaturated group in various dyes are responsible for absorption in the UV or visible region is known as a chromophore. For example, C=C, C≡C, C=O, C≡N, N=N, NO_2 etc. However it is not necessary a chromophore imparts color by absorbing light in any wavelength region. It will only appreaed colored if it absorbs light in visible regions (400-800 nm).Chromophores like C=C or C≡C having π electrons undergo π → π* transitions and those having both π and non-bonding electrons, e.g., C=O, C≡N or N=N, undergo π → π*, n → π* and n → σ* transitions. There are three main components such as chromogen, chromophore and auxochrome which are responsible for the color of any organic dyes and holding the electronic properties. The chromogen is a chemical compound that is either colored or could be made colored by the attachment of suitable substituent. The chromophore and the auxochrome(s) are also part of the chromogen [4].
As, there are many of many dyes presently involved in day to day life. But, here we are

focusing on some specific organic dyes of greatest applications for human as drug delivery, cancer therapy and staining purposes [5]. Auramine O is a diarylmethane dye used as a fluorescent stain. In its pure form, Auramine O appears as yellow needle crystals. It finds numerous applications to stain acid-fast bacteria (e.g. Mycobacterium, where it binds to the mycolic acid in its cell wall) [6]. Rhodamine B is often used as a tracer dye within water to determine the rate and direction of flow and transport. It is used extensively in biotechnology applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA.Rhodamine B is used in biology as a staining fluorescent dye, sometimes in combination with auramine O, as the auramine-rhodamine stain to demonstrate acid-fast organisms, notably Mycobacterium [7,8].Methylene blue, also known as methylthioninium chloride, is a widely used as photosensitizer and finds greater application in improved cancer therapy by photodynamic action. Previously it also been used for cyanide poisoning and urinary tract infections, but this use is no longer recommended [9].Nile blue (or Nile blue A) is a stain used in biology and histology. It may be used with live or fixed cells, and imparts a blue colour to cell nuclei. It may also be used in conjunction with fluorescence microscopy to stain for the presence of polyhydroxybutyrate granules in prokaryotic or eukaryotic cells. It is also used for histological staining of biological preparations. It highlights the distinction between neutral lipids (triglycerides, cholesteryl esters, steroids) which are stained pink and acids (fatty acids, chromolipids, phospholipids) which are stained blue [10]. Methyl orange is a pH indicator frequently used in titrations because of its clear and distinct colour change to yellow. Owing to its propertie of exhibiting different colors at different pH it has been used in titrations for acids.

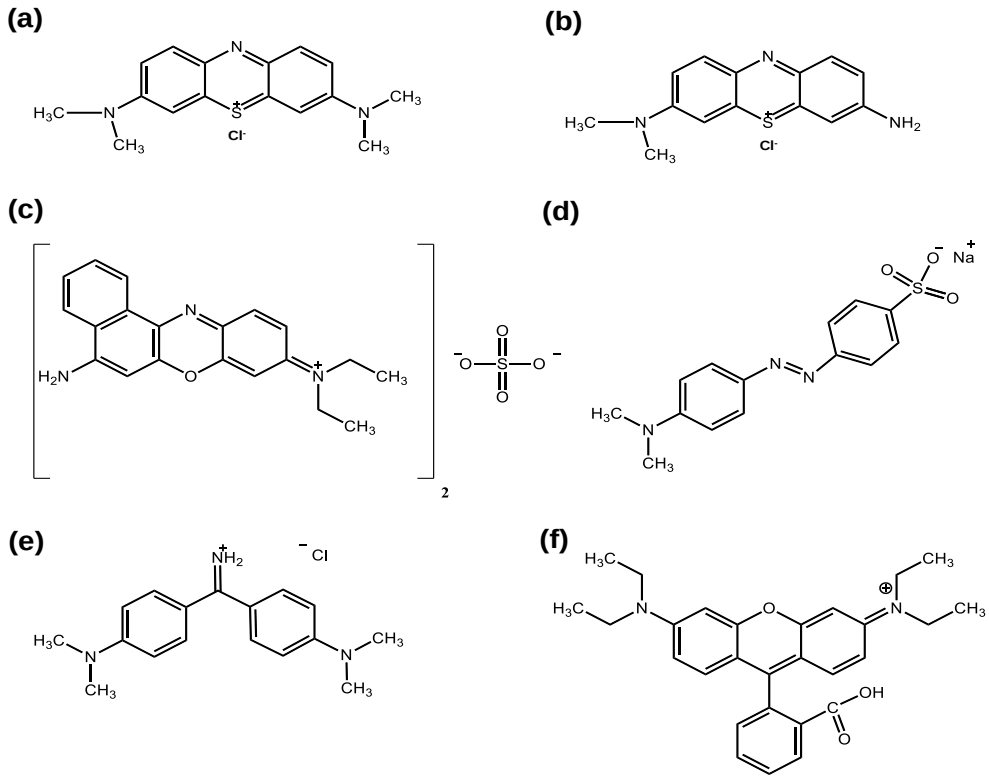

Fig. 1: Chemical structure of different organic dyes. (a) Methylene blue, (b) Azur II, (c) Nile blue sulfate, (d) Methyl orange, (e) Auramine O and (f) Rodamine

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibres, gases and surfaces can all be examined with a judicious choice of sampling technique. This technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the characteristics frequency of a vibration of a part of a sample molecule.The various factors like force constant of the bond, reduced mass of the compound and intensity of the incident radiations are responsible for the position and intensity of infrared modes. Factors such as combination and overtone bands, Fermi resonance, coupling and vibration– rotation bands can lead to changes in infrared spectra. Further, Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas by transforming signals using a mathematical parameter setup and shows spectra as a function of frequency. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range.

Hence, FTIR spectroscopy finds numerous applications in deducing the functional groups in various organic compounds. So, it can be inferred that properties of any dye mostly determined by the presence of functional group on that compound. So a critical investigation of the functional group of these dyes is severely important. This critical investigation can be easily carried out using FTIR spectroscopy [11]. In the present study, we have reported the FTIR spectroscopic analysis of various organic dyes like methylene blue, Azur II, nile blue sulfate, methyl orange, auramine O and rodamine B. A precise functional group characterization of these dyes has been reported by employing the FTIR spectrum in ATR mode. This spectroscopic analysis can be beneficial for accessing the binding tendency of these dyes with various nanosystems for effective and enhanced applications. This study will provides a basis for effective binding of various dyes by presence of specific functionality and can be useful for numerous applications like photodynamic therapy, drug delivery, enhanced cancer therapy, bacterial staining and water remediation applications.

Materials and Methods

Methylene blue, Nile blue sulfate, Azur II, Rodamine B, Auramine O and Methyl orange have been purchased from Sigma Aldrich (USA). Double distilled Milli Q DNA free, RNA free water obtained from Milli Q water assembly. All other chemicals were obtained locally and were of analytical reagent grade. Fourier transform infrared (Cary 630 FTIR, Agilent, USA) in ATR mode was employed to collect the spectra of samples to identify the presence of functional groups. The optical properties were studied using UV−Vis spectrophotometer (Cary 5000 UV-VIS-NIR, Agilent, USA).

Result and Discussion

The presence of different functional group on different organic dyes have been carried out using FTIR spectroscopic tool using ATR mode. **Figure 2** showing the FTIR spectrum of the nile blue sulfate dye. The presence of sharp peak around 3000 cm^{-1} is attributed due to the presence of N-H stretching of the amine salt group. Also the presence of strong bend at 1650 cm^{-1} due to the N-H bending confirms the presence of the NH_2 group in the compound.

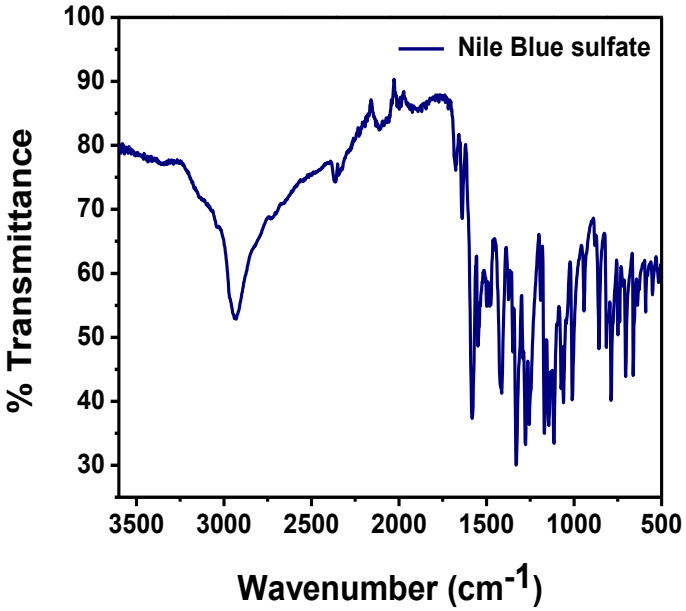

Fig. 2: FTIR spectrum of Nile blue sulfate in ATR mode.

Further the presence of strong peak around 1650 cm^{-1} may be attributed to the presence of C=N stretch and signifies the presence of imine linkage in the cytoskeleton of the compound. The appearance of peaks around 1050 cm^{-1} and 1250 cm^{-1} may be attributed to the presence of C-O stretching as well as C-N stretching respectively.

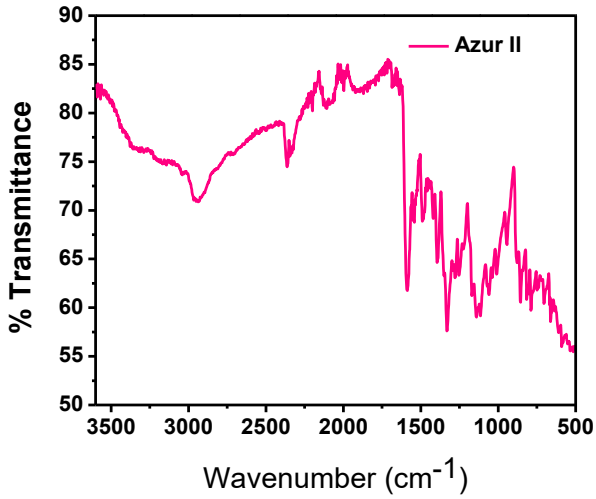

Fig. 3: FTIR spectrum of Azur II in ATR mode.

Figure 3 showing the FTIR spectrum of the Azur II dye. The presence of amino group can be investigate by observing the sharp peak around 3000 cm^{-1} which is due to the presence of N-H stretching of the amine group. Further, the appearance of the strong bend at 1640 cm^{-1} attributes towards N-H bending which confirms the presence of the substituted amine group in the compound. The appearance of strong peak around 1720 cm^{-1} signifies the presence of C=S group. Also, the presence of peak around 1650 cm^{-1} may be attributed to the presence of C=N imine linkage in the compound as this strong bend is due to the imine stretching. Further, the peaks around 1250 cm^{-1} attributed towards the C-N stretching of the amine groups in the side chains.

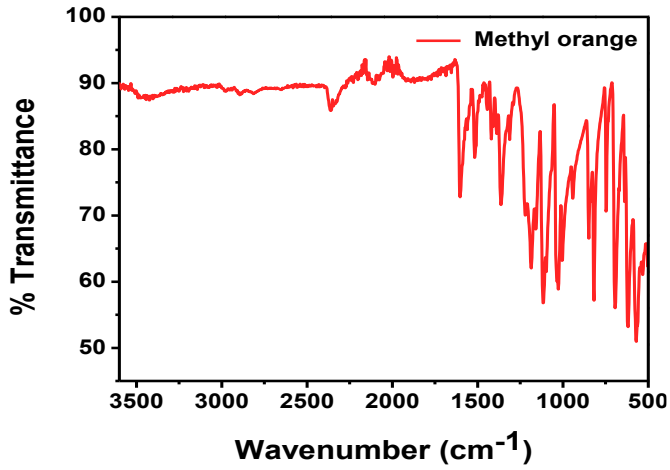

Fig.4: FTIR spectrum of methyl orange in ATR mode.

The FTIR spectrum of methyl orange has been investigated using ATR mode. In Figure 4, the presence of strong peak around 1280 cm^{-1} may be attributed due to the presence of C-S bond of the sulfonate group in the compound. The appearance of bands around 1400 cm^{-1} are due to the presence of the trans isomer of the compound and are confirming the presence of the azo group in the compound. Again the presence of weak band around 3300 cm^{-1} showing the presence of amine group. Also, presence of strong peaks around 1350 cm^{-1} due to C-N stretch of aromatic amine confirms the presence of the aromatic amine in the compound. Also, appearance of peak around 1290 cm^{-1} signifies the presence of S-O in the compound. Further, the presence of weak signal around 2000 cm^{-1} may be attributed to keteamine group in the compound. The appearance of peaks around 1050 cm^{-1} and 1250 cm^{-1} may be attributed to the presence of S-O stretching as well as C-N stretching respectively.

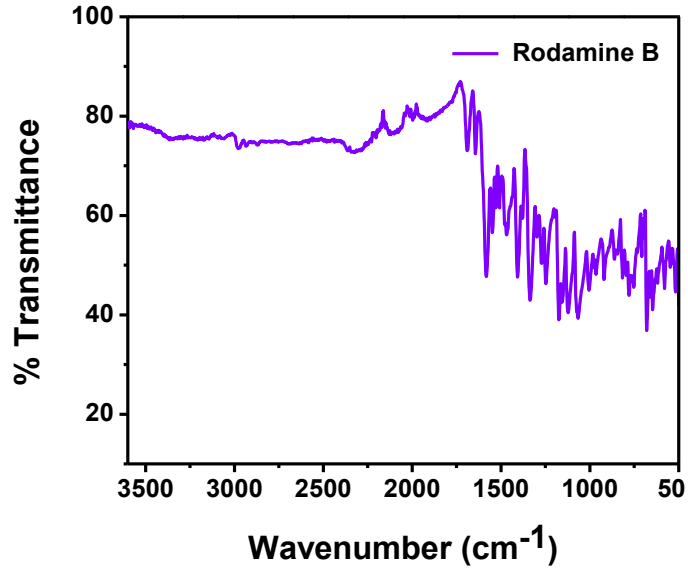

Fig. 5: FTIR spectrum of Rodamine B in ATR mode.

Fig 5 showing the FTIR spectrum of the rodamine B dye in ATR mode. The presence of strong band around 1720 cm^{-1} is attributed to the presence of carbonyl group in the compound. Further, appearance of broad peak around 3200 cm^{-1} is due to the presence of –OH group of the carboxylic acid. The appearance of peaks around 1050 cm^{-1} and 1250 cm^{-1} may be attributed to the presence of C-O stretching as well as C-N stretching respectively. The presence of amino group can be seen by due to the peak around 3000 cm^{-1} which may be attributed due to the presence of N-H stretching of the amine group. Again, the appearance of the strong bend at 1640 cm^{-1} attributes towards N-H bending due to the substituted amine group in the compound. Again, the appearence of peak around 1650 cm^{-1} attributed to the presence of C=N imine linkage in the compound. Further, the peaks around 1250 cm^{-1} attributed towards the C-N stretching of the amine groups in the side chains.

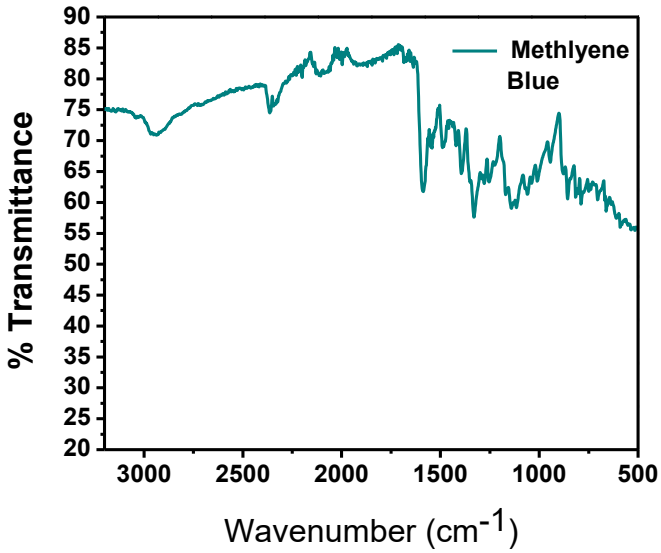

Fig.6: FTIR spectrum of Methylene blue in ATR mode.

Fig 6is showing the FTIR spectrum of Methylene blue dye employed in ATR mode. As methylene blue and Azur II belongs to the same family of compound. Hence, there is not much difference in the FTIR spectrum of both compounds. The presence of peaks around 3000 cm^{-1}, 1645 cm^{-1}, 1720 cm^{-1} and 1650 cm^{-1} attributed to the presence of N-H stretch, N-H bending, C=S stretch and C=N respectively.

Conclusion

The wide applications of organic dyes in drug delivery, cancer therapy, photodynamic and photothermal therapy and for various biological staining purposes makes it critically important to investigate the functional group of the compounds. In the present study, we have reported the FTIR spectroscopic analysis of various organic dyes like methylene blue, Azur II, nile blue sulfate, methyl orange, auramine O and rodamine B. This study will provides the basis for further application of these dyes by chemical modifications and surface- structural design with other nanosystems.

Acknowledgement

Authors would like to acknowledge principal of Pt. N. R. S. Govt. College for providing constant support and encouragement. Also, Mr. Rahul Sharma from CSIR-National Physical Laboratory, New Delhi has also been acknowledged for accessing the FTIR and UV-Visible facilities.

References

1. Kirk-Othmer (1998a) Encyclopedia of chemical technology fourth edition, vol 17. Wiley, New York.

2. Singh HB, Bharati KA (2014) Handbook of natural dyes and pigments. Woodhead Publishing, New Delhi.
3. Asiri AM (2001) organometallic dyes: Part 1. Synthesis of orange to cyan dyes based on donor-conjugated-acceptor-chromogenes using ferrocene as the donor group. Appl Organomet Chem 15:907–915.
4. Carmen Z, Daniela S (2012) Textile organic dyes—characteristics, polluting effects and separation/elimination procedures from industrial effluents—a critical overview. In: Puzyn T (ed) Organic pollutants ten years after the stockholm convention—environmental and analytical update. InTech Press, Crotia, pp 55–86.
5. Kommareddi, S.; Abramowsky, C. R.; Swinehart, G. L.; Hrabak, L. Nontuberculous Mycobacterial Infections: Comparison of the Fluorescent Auramine-O and Ziehl-Neelsen Techniques in Tissue Diagnosis. Hum. Pathol. 1984, 15 (11), 1085–1089.
6. Truant, J. P.; Brett, W. A.; Thomas, W. Fluorescence Microscopy of Tubercle Bacilli Stained with Auramine and Rhodamine. Henry Ford Hosp. Med. Bull. 1962, 10, 287–296.
7. Hooja, S.; Pal, N.; Malhotra, B.; Goyal, S.; Kumar, V.; Vyas, L. Comparison of Ziehl Neelsen & Auramine O Staining Methods on Direct and Concentrated Smears in Clinical Specimens. *Indian J. Tuberc.* 2011, *58* (2), 72–76.
8. Hendry, C.; Dionne, K.; Hedgepeth, A.; Carroll, K.; Parrish, N. Evaluation of a Rapid Fluorescent Staining Method for Detection of Mycobacteria in Clinical Specimens. *J. Clin. Microbiol.* 2009, *47* (4), 1206–1208.
9. Tardivo, J. P.; Del Giglio, A.; de Oliveira, C. S.; Gabrielli, D. S.; Junqueira, H. C.; Tada, D. B.; Severino, D.; de Fátima Turchiello, R.; Baptista, M. S. Methylene Blue in Photodynamic Therapy: From Basic Mechanisms to Clinical Applications. *Photodiagnosis Photodyn. Ther.* 2005, *2* (3), 175–191.
10. Dunnigan, M. G. The Use of Nile blue Sulphate in the Histochemical Identification of Phospholipids. *Stain Technol.* 1968, *43* (5), 249–256.
11. Amenabar, I.; Poly, S.; Nuansing, W.; Hubrich, E. H.; Govyadinov, A. A.; Huth, F.; Krutokhvostov, R.; Zhang, L.; Knez, M.; Heberle, J.; et al. Structural Analysis and Mapping of Individual Protein Complexes by Infrared Nanospectroscopy. Nat. Commun. 2013, 4, 2890