ejpmr, 2015,2(3), 417-425

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

SUDAN DYES AS LIPID SOLUBLE ARYL-AZO NAPHTHOLS FOR MICROBIAL STAINING

Rutvi Patel, Clive Dadida, Kinsuk Sarker and Prof. Dr. Dhrubo Jyoti Sen*

Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

Article Received on 12/03/2015 **Article Revised on 03/04/2015** **Article Accepted on 27/04/2015**

***Correspondence for Author**

Prof. Dr. Dhrubo Jyoti Sen

Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

ABSTRACT

Sudan stains and Sudan dyes are synthetic organic compounds that are used as dyes for various plastics and are also used to stain sudanophilic biological samples, usually lipids. Sudan I, Sudan II, Sudan III, Sudan IV, Oil Red O and Sudan Black Bare important members of this class of compounds. Sudan dyes have high affinity to fats; therefore they are used to demonstrate triglycerides, lipids and lipoproteins. Alcoholic solutions of Sudan dyes are usually used; however pyridine solutions can be used in some situations as well. Fat soluble Sudan dyes can easily stain the lipoprotein & phospholipid layer of bacterial cell to produce gram staining of gm+ve and gm-ve bacteria.

KEYWORDS: Sudan stains, Aryl-azo naphthols, Lipids, Triglycerides, Lipoproteins, Phospholipids, Gram Staining.

Sudan stain test is often used to determine the level of fecal fat to diagnose steatorrhea. A small sample is dissolved in water or saline, glacial acetic acid is added to hydrolyze the insoluble salts of fatty acids, a few drops of alcoholic solution of Sudan III are added, the sample is spread on a microscopic slide and heated twice to boil. Normally a stool sample should show only a few drops of red-orange stained fat under the microscope. The method is only semi-quantitative but, due to its simplicity, it is used for screening.[1]

Since they are characteristically "oil-and fat-soluble," Sudan dyes are also useful for dying plastics and fabrics. Sudan dyes I-IV and Sudan Red G consist of aryl-azo-substituted naphthols. Such compounds are known to exist as a pair of tautomers.

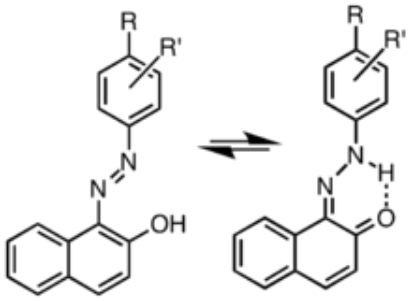

Figure-1: Tautomerism in aryl-azo naphthol

Sudan I (1-phenylazonaphth-2-ol) ($C_{16}H_{12}N_2O$) (also commonly known as **CI Solvent Yellow 14** and **Solvent Orange R**), is an organic compound, typically classified as an azo dye. It is an intensely orange-red solid that is added to colorize waxes, oils, petrol, solvents and polishes. Sudan I has also been adopted for colouring various foodstuffs, especially curry powder and chili powder, although the use of Sudan I in foods is now banned in many countries, because Sudan I, Sudan III and Sudan IV have been classified as category 3 carcinogens (not classifiable as to its carcinogenicity to humans) by the International Agency for Research on Cancer. Sudan I is still used in some orange-coloured smoke formulations and as a colouring for cotton refuse used in chemistry experiments.

Figure-2: Sudan I

Sudan I is genotoxic. It is also carcinogenic in rats. Comparisons between experimental animals and human Cytochrome P450 (CYP) strongly suggest animal carcinogenicity data can be extrapolated to humans. Sudan I is also present as an impurity in Sunset Yellow, which is its disulfonated water-soluble version.[2]

Sudan II (1-(2,4-dimethylphenyl)azonapthalen-2-ol) ($C_{18}H_{16}N_2O$) is a lysochrome (fat-soluble dye) azo dye used for staining of triglycerides in frozen sections and some protein bound lipids and lipoproteins on paraffin sections. It has the appearance of red powder with melting point 156–158°C and maximum absorption at 493(420)nm.

Figure-3: Sudan II

Its other names are **Solvent Orange 7**, **C.I. Solvent Orange 7** and **C.I. 12140**. It is also known as: A.F. red No. 5, Aizen food red No. 5, Brasilazina oil scarlet 6G, Brilliant oil scarlet B, Calco oil scarlet BL, Ceres Orange RR, Cerisol scarlet G, Cerotinscharlach G, Ext. D and C red No. 14, Extract D and C red No. 14, Fast oil Orange II, Fat scarlet 2G, FD and C red 32, FettOrange B, Grasan Orange 3R, Lacquer Orange VR, Motirot G, Oil Orange KB, Oil red GRO, Orange oil KB, Orange RR, Ponceau à l'huile, Pyronalrot R, Red B, Red No. 5, Resin scarlet 2R, Resoform Orange R, Rot B, Somalia Orange 2R, Soudan II, Sudan AX, Sudan Orange, Sudan red, Sudan scarlet 6G, Sudan X, Waxakol vermilion L. In industry, it is used to color nonpolar substances like oils, fats, waxes, greases, various hydrocarbon products and acrylic emulsions.[3]

Sudan III (1-(4-(phenyldiazenyl)phenyl)azonaphthalen-2-ol) ($C_{22}H_{16}N_4O$) is a lysochrome (fat-soluble dye) diazo dye. It is structurally related to azobenzene.

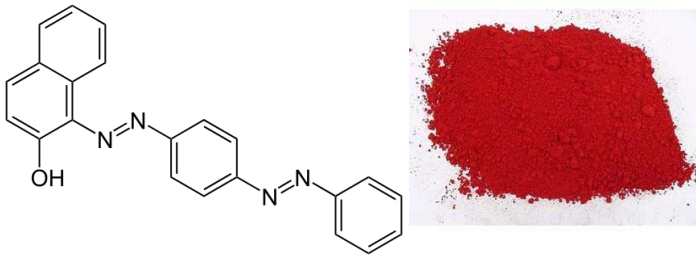

Figure-4: Sudan III

It is used to color nonpolar substances like oils, fats, waxes, greases, various hydrocarbon products, and acrylic emulsions. Its main use is as a fuel dye in the United States of America mandated by the IRS to distinguish low-taxed heating oil from automotive diesel fuel and by the EPA to mark fuels with higher sulfur content; it is a replacement for Solvent Red 26 with better solubility in hydrocarbons. The concentration required by IRS is a spectral equivalent of 3.9 pounds per 1000 barrels, or 11.13 mg/l, of Solvent Red 26 in solid form; the concentrations required by EPA are roughly 5 times lower. It is also used to dye

some hydraulic fluids and some other hydrocarbons, predominantly gasoline. Sudan III is a dye used for Sudan staining. Similar dyes include Oil Red O, Sudan IV and Sudan Black B. They are used for staining of triglycerides in frozen sections, and some protein bound lipids and lipoproteins on paraffin sections. It has the appearance of reddish brown crystals and a maximum absorption at 507(304)nm.

Its other names are **Sudan Red BK**, **Fat Ponceau G**, **Cerasin Red**, **C.I. 26100**, **Solvent Red 23**, **Sudan Red**, **Sudan Red III**, **Sudan V**, **Sudan Red B**, **Sudan G**, **Scarlet B** and **Tony Red**.

Sudan I, Sudan III, and Sudan IV have been classified as category 3 carcinogens by the International Agency for Research on Cancer.[4]

Sudan IV (1-(2-methyl-4-(2-methylphenyldiazenyl)phenyl)azonapthalen-2-ol) ($C_{24}H_{20}N_4O$) is a lysochrome (fat-soluble dye) diazo dye used for the staining of lipids, triglycerides and lipoproteins on frozen paraffin sections. It has the appearance of reddish brown crystals with melting point 199°C and maximum absorption at 520(357)nm.

Sudan IV is one of the dyes used for Sudan staining. Similar dyes include Oil Red O, Sudan III and Sudan Black B. Staining is an important biochemical technique, offering the ability to visually qualify the presence of the fatty compound of interest without isolating it. For staining purposes Sudan IV can be made up in propylene glycol. This is used in the dye saturated in isopropyl alcohol, 95% ethanol or 0.05% by weight in acetone:ethanol:water (50:35:15). The idea is to use a moderately apolar solvent to solubilize the dye allowing it to partition into the highly apolar fat without the solvent solubilizing the fat to be stained.

Figure-5: Sudan IV

Sudan I, Sudan III, and Sudan IV have been classified as category 3carcinogens by the International Agency for Research on Cancer. In its purified form it is called **Biebrich scarlet R**, which should not be confused with the water-soluble Biebrich scarlet.

In industry, it is used to color nonpolar substances like oils, fats, waxes, greases, various hydrocarbon products, and acrylic emulsions. Sudan IV is also used in United Kingdom as a fuel dye to dye lower-taxed heating oil; because of that it is also known as **Oil Tax Red**. As a food dye, Sudan IV is considered an illegal dye, mainly because of its harmful effect over a long period of time, as it is a carcinogen. It was ruled unsafe in the 1995 food safety regulations report.[5]

Sudan Black B (2,2-dimethyl-1,3-dihydroperimidin-6-yl)-(4-phenylazo-1-naphthyl)diazene) ($C_{29}H_{24}N_6$) is a nonfluorescent, relatively thermostable lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections. It has the appearance of a dark brown to black powder with maximum absorption at 596-605 nm and melting point 120–124°C. It stains blue-black.

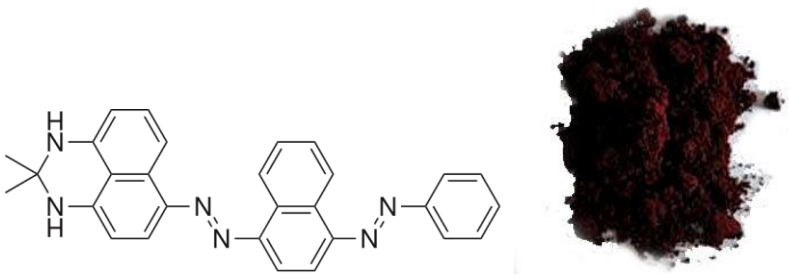

Figure-6: Sudan Black B

Sudan Black B is one of the dyes used for Sudan staining. Similar dyes include Oil Red O, Sudan III, and Sudan IV. Sudan Black B can be used to stain some other materials than the other Sudan dyes, as it is not so specific to lipids.

A use of Sudan Black B is in fingerprint enhancement. It is useful for detecting fats that are contaminated with oil and grease. In differentiating haematological disorders Sudan black will stain myeloblastsbut not lymphoblasts.

Sudan Black is formed by coupling of diazotized 4-phenylazonaphthalenamine-1 with 2,3-dihydro-2,2-dimethyl-1H-pyrimidine. Therefore the main product expected was 2,3-dihydro-2,2dimethyl-6-[(4-phenylazo-1-naphthalenyl)-azo]-1*H*-pyrimidine. However the dye resulting from the above reaction product actually contains many, up to 42 colored and

colorless by products that can be fractionated. The two major products were blue in color confirmed by various chromato-graphic (TLC and column etc.) separations and spectroscopic (IR, NMR, Mass) identification were named SSB-I & SSB-II (Rf values of 0.49 and 0.19 in thin Layer Chromatography). The above described product indeed turned out to be SSB-II which comprises up to 60% of the mixture, and the SSB-I was 2,3-dihydro-2,2dimethyl-4-[(4-phenylazo-1-naphthalenyl)-azo]-1*H*-pyrimidine.[6]

Sudan Red G (2-methoxybenzenazo-β-naphthol) ($C_{17}H_{14}N_2O_2$) is a yellowish red lysochrome azo dye. It has the appearance of an odorless reddish-orange powder with melting point 225°C. It is soluble in fats and used for coloring of fats, oils and waxes, including the waxes used in turpentine-based polishes. It is also used in polystyrene, cellulose, and synthetic lacquers. It is insoluble in water. It is stable to temperatures of about 100-110°C. It was used as a food dye. It is used in some temporary tattoos, where it can cause contact dermatitis. It is also used in hair dyes. It is a component of some newer formulas for red smoke signals and smoke-screens, together with Disperse Red 11.

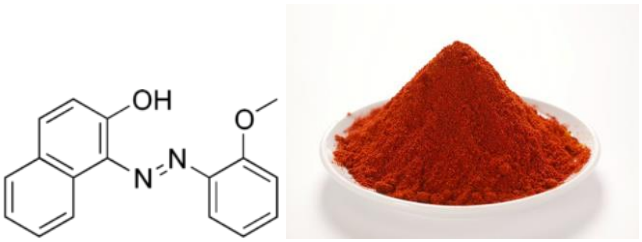

Figure-7: Sudan Red G

There are various names for Sudan Red G, including **Brilliant Fat Scarlet R**,**C.I. Food Red 16**, **C.I. Solvent Red I**, **C.I. 12150**, **Ceres Red G**, **Fat Red BG**, **Fat Red G**. **Lacquer Red V2G**, **Oil Pink**, **Oil Scarlet 389**, **Oil Vermilion**,**Oil Red G**, **Oleal Red G**, **Plastoresin Red FR**, **Red GD**, **Resinol Red G**,**Silotras Red TG**, **Solvent Red 1**, **Sudan R**, and **amethoxybenzenazo-β-naphthol** (**MBN**). According to European Food Safety Authority, Sudan Red G is considered genotoxic and/or carcinogenic.[7]

Oil Red O (1-(2,5-dimethyl-4-(2-5-dimethylphenyl)phenyldiazenyl)azonapthalen-2-ol) (**Solvent Red 27**, **Sudan Red 5B**, **C.I. 26125**, $C_{26}H_{24}N_4O$) is a lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections. It has the appearance of a red powder with maximum absorption at 518 (359)nm.[8]

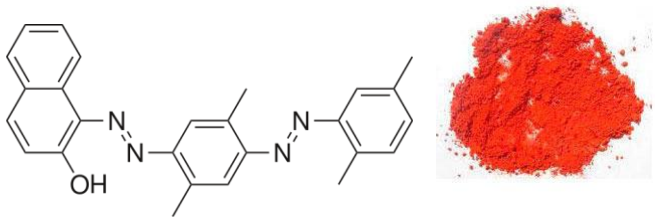

Figure-8: Oil Red O

Oil Red O is one of the dyes used for Sudan staining. Similar dyes include Sudan III, Sudan IV and Sudan Black B. The staining has to be performed on fresh samples, as alcohol fixation removes most lipids. Oil Red O largely replaced Sudan III and Sudan IV, as it provides much deeper red color and the stains are therefore much easier to see. In pyrotechnics, Oil Red O is used in some compositions of red colored smokes. It is also used for making dyes. When staining, Oil Red O can make fat more visible in various cuts in pathology.

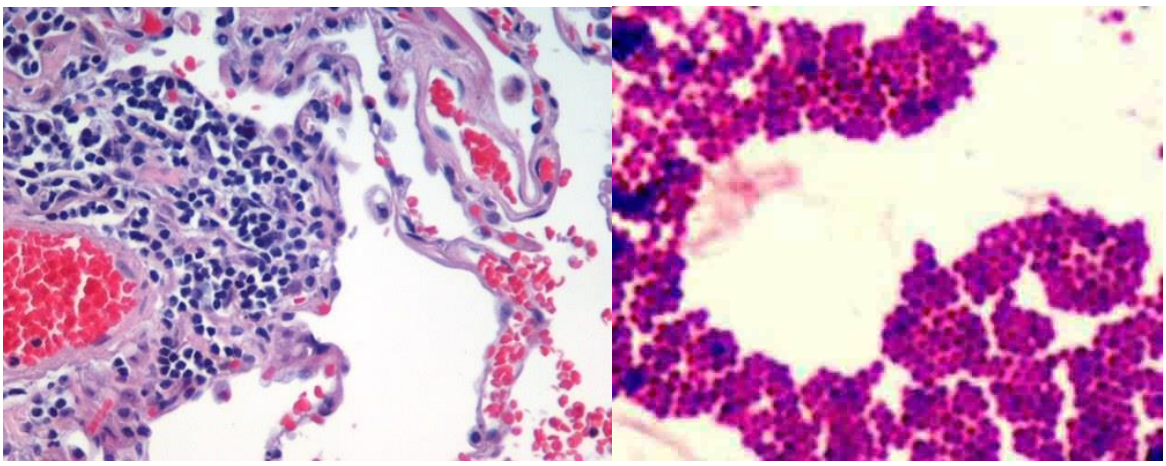

Figure-9: Gram staining of bacteria

It is also used in a technique (the method is called as the dye: Oil Red O), discovered in 2004 by Alexandre Beaudoin, for staining latent fingerprints. This technique allows the development of latent fingerprints on porous exhibits (such as paper, cardboard, etc.) that are dry or wet. It mainly targets fat deposits on the surface of porous exhibits. It is a non-destructive technique (which does not destroy the exhibit and doesn't prevent the use of other techniques). It is a safe alternative to the Physical Developer method and is also used in sequence with other methods of fingerprints development.[9]

Figure-10: Histopathological Examination in Bacterial Staining

CONCLUSION

One of the most widely used microbiological techniques, involving the staining of fixed cells of microorganisms with special dyes. The cells are applied with a loop to a drop of water on a slide or cover glass. After the cell suspension dries, the preparation is fixed with special fluids. To highlight the morphology of cells, alcohol solutions of the basic dyes methylene blue, gentian violet, or fuchsine or the acidic dyes erythrosine or eosin are used. The spores or flagella of bacteria and the mucous capsule of some microorganisms are stained using special techniques. Gram's method of diagnostic staining is widely used. Coccal and spore forms of bacteria and yeasts are Gram-positive and stain blue. Many bacteria that do not form spores are Gram-negative and stain red.

Certain dyes or chemical reagents are used for microscopic chemical analysis, that is, to detect organic compounds in cells. For example, intracellular lipids are stained black by osmic acid anhydride and red by Sudan. Red metachromatin granules precipitate in vacuoles with neutral red stain. Lugol's solution stains starch cinnamon-brown and granulose blue. Acid-resistant bacteria, for example, mycobacteria (specifically, the causative agents of leprosy and tuberculosis in man and animals), are stained red by fuchsine and do not lose their color when treated with sulfuric acid. A common procedure is vital staining, that is, the staining of non fixed, living microbial cells. Fluorescent dyes, for example, acridine orange,

in combination with fluorescence microscopy are used to distinguish living cells, which stain green, from dead ones, which stain red. Another widely used technique is to combine fluorescent dyes with serum that contains antibodies to a certain microbial species: only the cells of the given species will fluoresce under the microscope. This method permits the researcher to identify a microorganism in the soil or a pathogenic microbe in the intestine, blood, or sputum of a patient without resorting to culture techniques, which are considerably more time-consuming.

REFERENCES

1. Booth, Gerald (2000). *Dyes, General Survey*. Wiley-VCH. doi:10.1002/14356007.a09_073.
2. Larsen, John Chr. "Legal and illegal colors" Trends in Food Science & Technology (2008), 19(Suppl. 1), S60-S65.
3. Refat NA, Ibrahim ZS, Moustafa GG, Sakamoto KQ, Ishizuka M, Fujita S (2008). "The induction of cytochrome P450 1A1 by sudan dyes". *J. Biochem. Mol. Toxicol.* 22 (2): 77–84.
4. Stiborová M, Martínek V, Rýdlová H, Hodek P, Frei E (2002). "Sudan I is a potential carcinogen for humans: evidence for its metabolic activation and detoxication by human recombinant cytochrome P450 1A1 and liver microsomes". *Cancer Res.* 62 (20): 5678–84.
5. Refat NA, Ibrahim ZS, Moustafa GG, Sakamoto KQ, Ishizuka M, Fujita S (2008). "The induction of cytochrome P450 1A1 by Sudan dyes". *J. Biochem. Mol. Toxicol.* 22 (2): 77–84.
6. A. G. W. Lansink (1968). "Thin layer chrornatography and histochemistry of Sudan Black B". 16 (1): 68-84.
7. Beaudoin, A (2004). "New technique for revealing latent fingerprints on wet, porous surfaces: Oil Red O." *Journal of Forensic Identification*, 54 (4): 413-421.
8. Rawji, A. and Beaudoin, A (2006). "Oil Red O versus Physical Developer on wet papers: a comparative study". *Journal of Forensic Identification*, 56 (1): 33-54.
9. Guigui, K. and Beaudoin, A (2007). "The use of Oil Red O in sequence with other methods of fingerprint development". *Journal of Forensic Identification*, 57 (4): 550-581.