Review Article

ANALYTICAL, BIOCHEMICAL AND SYNTHETIC APPLICATIONS OF *Para*-DIMETHYLAMINOBENZALDEHYDE

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ABSTRACT

Para-dimethylaminobenzaldehyde (Ehrlich's reagent) has found usefulness in a wide range of applications from analytical, biochemical to synthetic procedures and processes alongside other miscellaneous applications. The unique structural feature of containing a *para*-dialkylamino substituent to an aldehyde moiety renders it highly reactive towards a wide range of compounds and this has made it useful as a condensation reagent, oxidizable and reducible reactant in many reactions. This paper reviews the diverse applications of DMAB spanning over a century with a prospect that this compound will be more relevant in years to come in microbiology, chemical pathology, and organic synthesis as well as pharmaceutical and analytical chemistry. Its extensive synthetic pathway appears to be a demerit to its application and a simpler pathway must be proposed to continue to make the compound readily available.

Keywords: *Para*-dimethylaminobenzaldehyde (DMAB), Applications, Overview, Future prospects.

1.0 INTRODUCTION

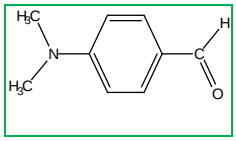
Aldehydes and ketones are simple compounds which contain a carbonyl group - a carbon-oxygen double bond. They are simple in the sense that they don't have other reactive groups like -OH or -Cl attached directly to the carbon atom in the carbonyl group[1]. Oxygen is far more electronegative than carbon and so has a strong tendency to pull electrons in a carbon-oxygen bond towards itself. One of the two pairs of electrons that make up a carbon-oxygen double bond is even more easily pulled towards the oxygen. That makes the carbon-oxygen double bond very highly polar. The slightly positive carbon atom in the carbonyl group can be attacked by nucleophiles. A nucleophile is a negatively charged ion (for example, a cyanide ion, CN^-), or a slightly negatively charged part of a molecule (for example, the lone pair on a nitrogen atom in ammonia, NH_3). During the reaction, the carbon-oxygen double bond gets broken. The net effect of all this is that the carbonyl group undergoes addition reactions, often followed by the loss of a water molecule. This gives a reaction known as addition-elimination or condensation[1].

Aldehydes also undergo other wide variety of chemical reactions, including polymerization. Their combination with other types of molecules produces the so-called aldehyde condensation polymers, which have been used in plastics such as Bakelite and in the laminate tabletop material Formica. Aldehydes are also useful as solvents and perfume ingredients and as intermediates in the production of dyes and pharmaceuticals. Certain aldehydes are involved in physiological processes. Examples are retinal (vitamin A aldehyde), important in human vision, and pyridoxal phosphate, one of the forms of vitamin B_6. Glucose and other so-called reducing sugars are aldehydes, as are several natural and synthetic hormones[2].

Aromatic aldehydes have enjoyed a wide range of applications in the development of synthetic, analytical and biochemical processes. One of such aromatic aldehyde that has enjoyed the widest application is *para*-dimethylaminobenzaldehyde (DMAB). This paper reviews the practical applications that DMAB has been put into for over a century with a view to discovering the relevance of the compound and its future prospect.

2.0 DESCRIPTION OF DMAB

Para-Dimethylaminobenzaldehyde (I, II-3D structure) is a bifunctional aromatic skeleton possessing the aldehyde (CHO) *para* to an activating substituent dimethylamino group [$-N(CH_3)_2$]. It's other synonyms are 4-(dimethylamino) benzaldehyde, *p*-(dimethylamino)-benzaldehyde, 4-Dimethylamino benzaldehyde; Ehrlichovo; Ehrlich's Reagent; *p*-Formyl-N,N-dimethylaniline; p-DAB; N,N-Dimethyl-4-amino benzaldehyde; 4-Dimethylaminobenzene carbonal; 4-N,N-Dimethylamino benzaldehyde; N,N-Dimethyl-*p*-amino benzaldehyde; N,N-Dimethyl-4-aminobenzaldehyde.



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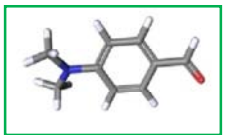

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DMAB is a white crystalline powder with a melting point of 72-75^{O}C and boiling point of 176-177^{O}C. Its molecular weight is 149.19 with a molecular formula of $C_9H_{10}NO$ and log P value of 1.8. It has two hydrogen bond acceptors with no hydrogen bond donor. It is stable under ordinary condition though light sensitive.

2.1 Synthesis of *p*-Dimethylaminobenzaldehyde

The most satisfactory method for the preparation of *p*-dimethylaminobenzaldehyde is the condensation of dimethylaniline, formaldehyde and *p*-nitrosodimethyl aniline, followed by hydrolysis, a method for which details have been perfected[3]. This synthetic pathway is shown in Figure 1. In a modification of this popular procedure, Ingvaldsen and Bauman[4] proposed that since an excess of the nitroso body is used, the second molecule of aldehyde is partially converted into the benzylidene body, the procedure is modified in several respects. The crude aldehyde is purified by distillation in a partial vacuum.

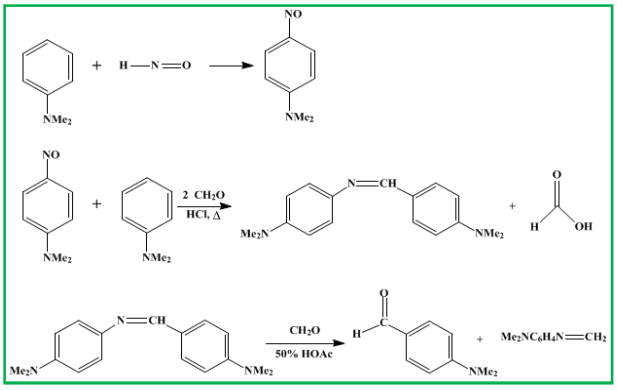


Figure 1: Synthetic pathway for DMAB

2.2 Peculiar Structural features of *p*-Dimethylamino benzaldehyde

DMAB possesses some peculiar structural features which might account for its applicability in a wide range of reactions and processes. 4-Dimethylaminobenzaldehyde is a *para*-substituted benzene derivative with a donor–aromatic–acceptor structure. This facilitates the contribution of two resonance forms to the electronic ground state (the neutral benzenoid structure and the zwitterionic quinonoid structure with a negative charge centred on the oxygen and a positive charge centred on the nitrogen)[5]. This electronic property coupled with three possible attachment points of DMAB to a surface provides a complex molecule-surface system. In an attempt to understand the DMAB-surface interaction, gold nanoparticles were utilized[6]. Absorption spectra collected for samples in aerobic and anaerobic conditions show that gold nanoparticles in an aqueous medium in the presence of dissolved oxygen catalyze the oxidation of 4-(dimethylamino)benzaldehyde to 4-(dimethylamino)benzoic acid in the pH range 2.6–11.7. GC and GC–MS results show that at acidic pH the only additional product is 4-(methylamino) benzoic acid whereas at basic pH a variety of by-products are formed. The 15 nm diameter gold particles employed are much larger than the gold particle diameters typically used for CO oxidation.

In a study of the lowest triplet of the intramolecular charge-transfer process using DMAB as a probe, the molecule was discovered not to have a pronounced charge transfer character in apolar solvents, since the rotation of the dimethylamino group will lead to important volume contraction[7].

Absorption and steady-state and time-resolved emission studies of DMAB in aqueous α-cyclodextrin (α-CD) solutions have been reported. The twisted intramolecular charge transfer (TICT) emission is extremely poor in pure water, but is greatly enhanced upon complexation with α -CD. The cavity size of α -CD is insufficient to encapsulate the entire fluorophore; rather it embeds DMAB only partially, keeping the dimethylamino group open in bulk water. The enhancement in the TICT emission is attributed to the reduced polarity provided by the CD environment[8]. The twisted motion is illustrated in Figure 2.

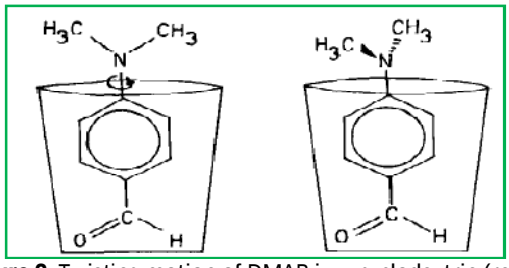


Figure 2: Twisting motion of DMAB in α-cyclodextrin (ref 8)

On forming semicarbazones, Trzesowska[9] showed on the basis of quantum mechanical calculations the availability of the imine nitrogen atom for bonding with molecular species thereby attesting to the wide applicability of these derivatives of DMAB. Thus, the imine nitrogen atom in compound of configuration E around CAN bond is likely not to be involved in semicarbazone–receptor interactions. Change of configuration leads to differences in the availability of electron donor atom. The rotation energy barrier is small and it is mostly related to energy of intramolecular hydrogen-bond break. The molecular structure of *p*-dimethylaminobenzaldehyde semi carbazone is shown in Figure 3.

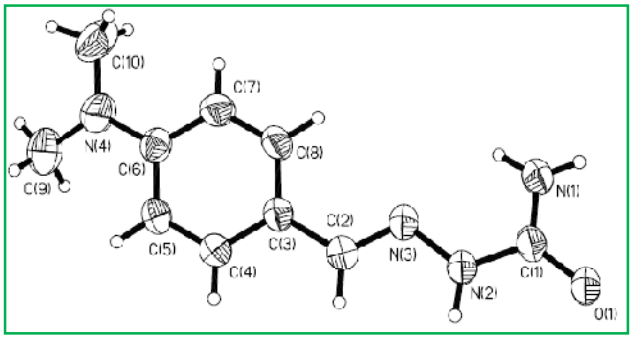


Figure 3: The molecular structure of *p*-dimethylaminobenzaldehyde semicarbazone. The displacement ellipsoids are drawn at 50% probability level (ref 9)

The heat capacities of p-dimethylaminobenzaldehyde were measured between 80 and 360 K by Meng et al [10] with a small sample automated adiabatic calorimeter. The thermodynamic parameters of solid-liquid phase transition were also obtained. The melting point, enthalpy and entropy of fusion of this compound were determined to be 346.15 K, 19.07 kJ mol^{-1} and 55.08 J mol^{-1} K^{-1}, respectively.

3.0 REPORTED APPLICATIONS OF P-DIMETHYLAMINOBENZALDEHYDE (DMAB)

Based on the above considered peculiar structural features of DMAB, several applications have been reported for this compound. This section of this review report chronicles the diverse applications of this important derivative of benzaldehyde.

3.1 Analytical Applications

The analytical applications of DMAB comprise its utilization for the determination of a wide range of substances notably among which are pharmaceuticals (inorganic and organic), bioactive substances and nanomaterials. In the applications of DMAB as an analytical reagent, use is made of its peculiar properties of forming condensation products, the ability of its aldehyde moiety being reduced to the alcohol or oxidized to the carboxylic acid.

3.1.1 Spectrophotometric methods

DMAB has been adopted solely or in combination with some reagents for the spectrophotometric analyses of pharmaceuticals.

A simple, accurate and sensitive spectrophotometric method has been developed and validated for determination of H_2-receptor antagonists: cimetidine, famotidine, nizatidine and ranitidine hydrochloride. The method was based on the oxidation of these drugs with cerium (IV) in presence of perchloric acid and subsequent measurement of the excess Ce (IV) by its reaction with DMAB to give a red colored product (λ_{max} at 464 nm). The decrease in the absorption intensity of the colored product (ΔA), due to the presence of the drug was correlated with its concentration in the sample solution. The results obtained by the proposed method were comparable with those obtained by the official methods[11]. Adegoke and Balogun also reported the spectrophotometric determination of three quinolones (ciprofloxacin, sparfloxacin and perfloxacin) using Ce (IV) with determination of excess oxidant by a reddish-brown colour formation with DMAB with good reproducibility and accuracy[12]. In both cases, the ability of excess unreacted Ce (IV) to oxidise DMAB is adopted.

In another application of DMAB, bopindolol was determined. In the proposed procedure, the determination of bopindolol using a sequential injection technique (SIA) with spectrophotometric detection at 560 nm is described. The new method of determination is based on the color reaction of the indole group in the molecule of bopindolol with DMAB (Ehrlich's reagent) in acidic medium with production of a violet water-soluble complex. The selectivity of the proposed method of determination was tested in the presence of seven interfering substances from the group of β-blockers with good results. The interference effect was observed only in the presence of pindolol. Obtained results were compared with conventional HPLC method both analytical techniques were in good agreement[13].

The color reaction of indole alkaloids with the DMAB/iron (III) chloride reagent suitable for the assay of various formulations of pharmaceuticals has been widely reported[14-20].

The spectrophotometric detection of 7-aminocephalo sporanic acid (7-ACA) is possible through imine formation of its amino functionality with DMAB [21]. This kind of derivatization was originally developed for the detection of 6-aminopenicillic acid[22, 23]. It has also been applied to the determination of 7-ACA[24, 25] and 7-amino desacetoxycephalosporanic acid[26, 27] after cleavage of their respective glutaryl derivatives.

The herbicide, isoproturon, has been determined spectrophotometrically using DMAB with good accuracy though methanolic NaOH was used for hydrolysis which was criticized by later studies[28].

DMAB has also been used in inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) which have been introduced and applied in analytical chemistry. In particular, DMAB is used in the preconcentration steps thus detection limits for ICP-MS and ICP-AES are at the very low ng/L and ng/ml, respectively, which may be useful for the determination of trace metals in real samples[29].

The rate of condensation of DMAB and other aromatic aldehydes with 5-*N*-benzoylamino-1, 3, 4-thiadiazole-2-acetonitrile has been studied and applied to the spectrophotometric determination of these aldehydes[30].

Another set of spectrophotometric applications of DMAB include the formation of Schiff bases. The Schiff bases are formed by the condensation of the aldehyde functional with amino donors and since this extends electronic conjugation many of the Schiff bases are brilliantly colored and are used for the spectrophotometric determination of the amino donors. Thus 6-APA and 6-ACA (Figure 4) have been determined spectrophotometrically in the presence of sodium dodecyl sulphate micelles *via* the formation of Schiff bases[31, 32]. Secnidazole has also been determined spectrophotometrically through Schiff base formation with DMAB with measurement made at 494 nm yielding good accuracy and reproducibility[33]. Hydralazine has also been determined using Schiff base formation with DMAB with good accuracy and reproducibility. The calibration range obtained with the DMAB method was particularly described as been better than previously adopted methods[34]. The Schiff base produced with hydralazine is shown in Figure 5. Ceftiofur has also been determined through a condensation reaction with DMAB [35]. A Schiff base formed between DMAB and sulphanilamide has been applied as acid-base indicator which represents a major departure from previously known applications of Schiff bases as they are often regarded as unstable to be so utilized in acid-base titrations[36]. Hydrazine derivatives supported on resin have been determined using DMAB in alkaline media[37]. Benzocaine is well known as an insoluble

substrate in water. Therefore, its physical and analytical treatments in the presence of surfactant may allow organic solvents to be replaced by aqueous surfactants solutions. The effect of the presence of anionic surfactant (sodium dodecyl sulphate, SDS) upon the condensation reaction of benzocaine with water insoluble *p*-dimethylaminobenzaldehyde (DMAB) in aqueous solution has been investigated extensively. The presence of $5x10^{-3}$ M SDS increases the reaction rate by 20 times in contrast to that of a solution in 20 % ethanol. A substantial increase in absorption band due to the formed Schiff base followed by a red shift has been observed. A great advantage in analytical application resulting from the presence of surfactant due to the formation of a charge transfer complex has been reported[38]. Chloramphenicol, an important antibacterial agent has also been determined *via* its formation of a Schiff base with DMAB[39].

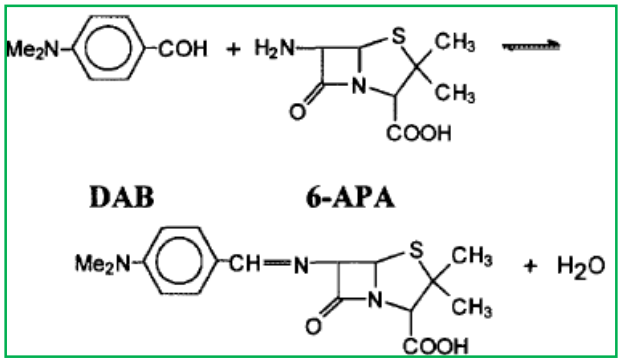


Figure 4: Determination of 6-APA with DMAB *via* Schiff base formation (ref 31)

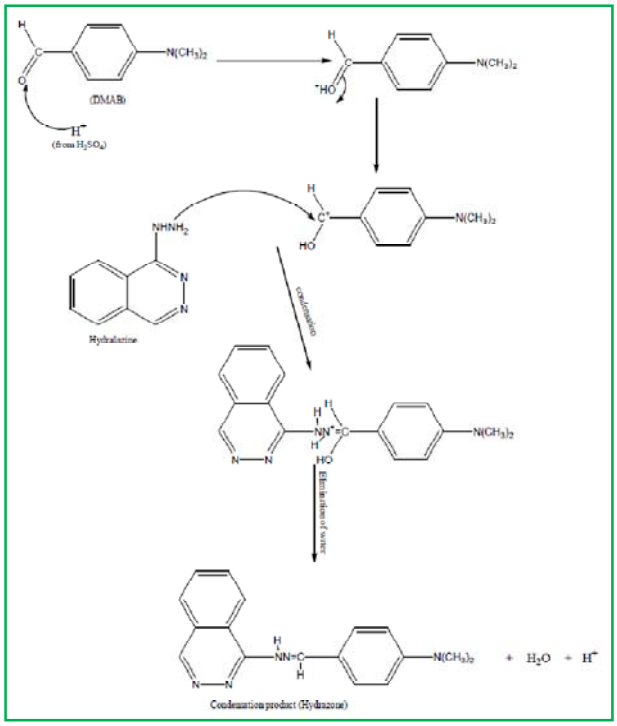


Figure 5: Schiff base formation between DMAB and hydralazine (ref 34)

A recent novel application of DMAB was its utilization as a coupling component to form azo adducts with reduced and diazotized nitroimidazoles. The method yielded results that were comparable with official methods and offered a promise for future applications to other diazotizable groups[40].

DMAB has also found practical relevance in the determination of environmental pollutants. The Person-Portable Analytical Kit (PPAK) was further expanded to allow the quantitative determination of primary amines. DMAB was used to analyse for hydrazine, aniline, *m*-nitroaniline and 2, 4-diaminotoluene in tap water, waste water and sea water at temperatures ranging from 10°C to 32°C[41].

A spectrophotometric method for the determination of total serotonin derivatives in the safflower (*Carthamus tinctorius* L.) seeds has been described. The determination is based upon a color reaction between serotonin derivatives and DMAB, which follows the electrophilic substitution reaction mechanism at the indole ring. The maximum absorption wavelength of the complex was determined at 625 nm[42]. The reaction occurring is shown in Figure 6.

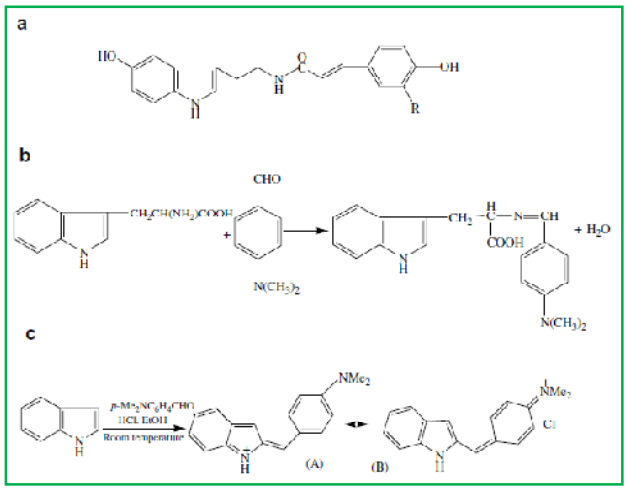


Figure 6: (a)-Chemical structures of feruloylserotonin (R=OCH_3) and *p*-coumaroylserotonin (R=H) in safflower seeds; (b)-condensation of tryptophan with Ehrlich's reagent and (c)-Reaction of indole ring derivatives with Ehrlich's reagent (ref 42).

3.1.2 Chromatographic methods

Para-Dimethylaminobenzaldehyde has also found relevance in some chromatographic techniques as a derivatization reagent either in spray solutions or for actual quantitation of molecules.

An HPTLC technique was developed for the measurement of urea in pharmaceutical formulations using DMAB [43]. The method involves HPTLC-densitometry and colorimetry following condensation of urea with DMAB with better results obtained using HPTLC. DMAB as a 10 % solution in HCl and acetone (1:4 v/v) has been used for the TLC detection of mycotoxins [44].

An adaptation of the Morgan-Elson for HPLC determination was proposed by Roden et al [45]. The Morgan–Elson method for quantitative *N*-

acetylhexosamine analysis is a two-step procedure comprising alkali treatment of the sugar and subsequent condensation of the resulting chromogens with DMAB to yield a colored product. The three predominant components, isolated by preparative HPLC, all gave a purple color on addition of DMAB, indicating that they were Morgan–Elson chromogens. The HPLC profile of alkali-treated *N*-acetylmannosamine was identical to that of the products generated from *N*-acetylglucosamine, as was expected because of the elimination of the asymmetry at C-2 during formation of the chromogens.

A liquid chromatographic (LC) method for the analysis of sulfamethazine (SMT) in complete swine and cattle feed was collaboratively studied. The method uses post-column derivatization with DMAB and detection at 450 nm. The authors recommended the method for AOAC INTERNATIONAL Official First Action status[46].

A method is described for the HPLC determination of phenylpropanolamine (PPA) based on precolumn derivatization with DMAB and elution from Phenomenex C-18 column with methanol–water and detection by spectrophotometry at 418 nm. Linear calibration was obtained with 9.4–46.9 μg mL^{-1} with a detection limit of 4.7 ng mL^{-1}. Vitamin B_{12} and rifampicin when present together with PPA separated completely and could be determined simultaneously[47].

3.1.3 Spot Tests and Test Strips

DMAB has also found usefulness in the spot tests or chemical functional group tests of some compounds either in solution form or as impregnated test strips. Validation procedures were described for 12 chemical spot tests including cobalt thiocyanate, Dille–Koppanyi, Duquenois–Levine, Mandelin, Marquis, nitric acid, DMAB, ferric chloride, Froehde, Mecke, Zwikker and Simon's (nitroprusside) for drugs of abuse. The validation procedures include specificity and limit of detection. Depending on the specificity of each color test, between 28 to 45 drugs or chemicals were tested in triplicate with each of the 12 chemical spot tests [48]. Likewise, gelatinous solidified layers of the photographic film were used for the immobilization of analytical reagents for detection and determination of reductants and primary aromatic amines. It was shown, that the films with immobilized iron(III)-Dipy or iron(III)-Phen complexes as test films for reductants and films with immobilized aldehydes (vanillin, DMAB) as the test films for primary aromatic amines can be used. The improving of reagents immobilization in the presence of sodium dodecyl sulphate micelles was obtained. The suggested test films for the determination of ascorbic acid, analgin (dipyrone), novocaine and streptocide in drugs were examined successfully[49].

3.2 Biochemical Applications

Erhlich's reagent derived its first set of applications in most biochemical applications. In particular, the use of the reagent has been the age-long method for the detection of indoles by microorganisms as well as the secretion of such compounds as tryptophan, serotonin and hydroxyproline from living tissues or cell cultures. This section therefore reviews the various applications of DMAB as a biochemical tool for analysis.

DMAB as presented in Kovacs' reagent is still the reagent of choice for the detection of indole produced by microorganisms and which is particularly useful for identifying the Enterobacteriaceae family. Indole is generated by reductive deamination from tryptophan *via* the intermediate molecule indolepyruvic acid. Tryptophanase catalyzes the deamination reaction, during which the amine ($-NH_2$) group of the tryptophan molecule is removed. Final products of the reaction are indole, pyruvic acid, ammonia (NH_3) and energy. Pyridoxal phosphate is required as a coenzyme.

Like many biochemical tests on bacteria, results of an indole test are indicated by a change in color following a reaction with an added reagent. Pure bacterial culture must be grown in sterile tryptophan or peptone broth for 24-48 hours before performing the test. Following incubation, add 5 drops of Kovacs' reagent (isoamyl alcohol, *p*-Dimethylaminobenzaldehyde, concentrated hydrochloric acid) to the culture broth. A variation on this test using Ehrlich's reagent (using ethyl alcohol in place of isoamyl alcohol, developed by Paul Ehrlich) is used when performing the test on non-fermenters and anaerobes. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow. A variable result can also occur, showing an orange color as a result. This is due to the presence of skatole, also known as methyl indole or methylated indole, another possible product of tryptophan degradation. Indole-Positive Bacteria that test positive for cleaving indole from tryptophan include: *Aeromonas hydrophilia*, *Aeromonas punctata*, *Bacillus alvei*, most *Citrobacter* sp., *Edwardsiella* sp., *Escherichia coli*, *Flavobacterium* sp., *Haemophilus influenzae*, *Klebsiella oxytoca*, *Proteus* sp. (not *P. mirabilis*), *Plesiomonas shigelloides*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Streptococcus faecalis* and *Vibrio* species. Bacteria which give negative results for the indole test include: *Actinobacillus* spp., *Aeromonas salmonicida*, *Alcaligenes* sp., most *Bacillus* sp., *Bordetella* sp., *Enterobacter* sp.,*Lactobacillus* spp., most *Haemophilus* sp., most *Klebsiella* sp., *Neisseria* sp., *Pasteurella haemolytica*, *Pasteurella ureae*, *Proteus mirabilis*, *Pseudomonas* sp., *Salmonella* sp., *Serratia* sp., *Yersinia* sp.[50, 51] Kitasato's discovery that *Escherichia coli* could be distinguished from *Klebsiella* species (*Aerobacter aerogenes*) by virtue of its ability to produce indole initiated the detection of indole production as an accepted analytical tool for the bacteriologist[52]. The use of *p*-dimethylaminobenzaldehyde for this purpose, first applied by Bohme[53], modified by Kovacs[54], and later refined by Gadebusch and Gabriel[55], has become the accepted method for the detection of this metabolite in cultures. Thus, Edwards and Ewing[56] and Kauffmann[57]

recommended the use of the simple Kovacs' test (p-DAB, direct). The Manual of Microbiological Methods [58] indicates Gore's[59] modification is more specific than the aforementioned Kovacs' test. The indole reaction is illustrated in Figure 7.

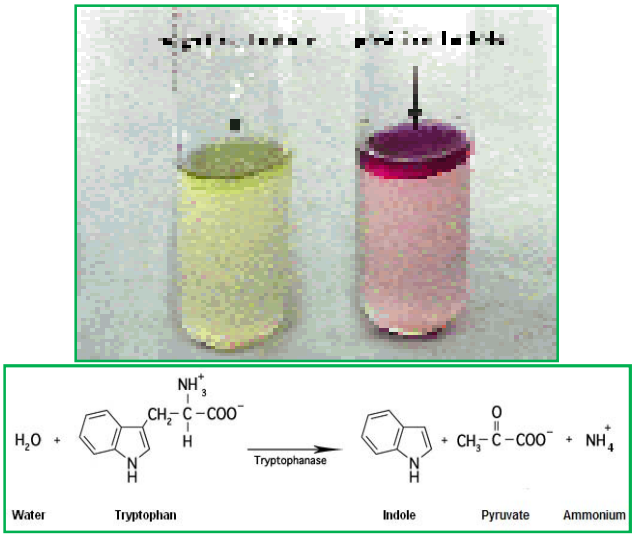


Figure 7: The indole reaction by bacteria

Hyaluronidase (HAase) activity has also been assessed by the use of DMAB. The colorimetric Morgan–Elson assay method, which is based upon the generation of a new reducing N-acetyl-D-glucosamine terminus with each cleavage reaction, is most widely employed but is yet insensitive. The colorimetric method was reinvestigated and established the fluorimetric Morgan–Elson assay for HAase activity, with the optimized tetraborate reagent. Human serum HAase was easily characterized it along with its optimum pH and kinetic parameters[60].

The kynurenine (KYN) pathway of tryptophan (TRP) degradation on gene transcription of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production in chicken interferon gamma (ChIFN-γ)-stimulated and non-stimulated chicken macrophage cell line HD11 has also been investigated spectrophotometrically using DMAB[61].

GM2 ganglioside, β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-]β-Gal-(1-4)-β-Glc-(1-1)-Cer, is the main ganglioside in the brain of Tay-Sachs patients. GM2 ganglioside was extracted from a Variant B Tay-Sachs human brain, purified to homogeneity of the oligosaccharide moiety by silica gel chromatography. It was further fractionated for the first time into the molecular species differing in the ceramide structures by reverse-phase flash chromatography. The GM2 ganglioside species were characterized by gas-chromatography, nuclear magnetic resonance spectroscopy, and mass spectrometry. Gangliosides on the TLC plate were made visible by spraying the plate with a DMAB followed by heating at 120°C for 20 min[62].

A natural agglutinin from the serum of Indian white shrimp (*Fenneropenaeus indicus*) was isolated and its chinitase activity was assessed using DMAB reagent. This assay involves release of N-acetylgalactosamine (GlcNAc) from colloidal chitin upon enzymatic hydrolysis by chitinase present in the sample (*Fenneropenaeus indicus* agglutinin), and then detection of released GlcNAc by *p*-dimethylaminobenzaldehyde (DMAB) reagent. The absorbance of the samples was read at 585 nm against the reagent blank[63].

Collagen integrity is often assayed by a measurement of the content of hydroxyproline and DMAB has also found wide applications in this regard[64]. The biochemical composition and biomechanical properties of articular cartilage from 53 human thumb carpometacarpal (CMC) joints from cadavers aged 20 to 79 years were measured and studied in normal, mildly fibrillated, and advanced osteoarthritic (OA) joints Colorimetric analysis was then used to measure the OH-Pro content when the residues were oxidized with chloramines-T reagent to form a compound that reacted with the DMAB[65].

Allografts of articular cartilage are both used clinically for tissue-transplantation procedures and experimentally as model systems to study the physiological behaviour of chondrocytes in their native extracellular matrix. Long-term maintenance of allograft tissue is challenging. The overall collagen content was assessed by measuring the *ortho*-hydroxyproline (OHP) content *via* dimethylamino benzaldehyde and chloramine T assay[66].

In measuring some common biochemical bone turnover parameters in menopausal women, Sachdeva *et al*[67] used 5 % solution of DMAB in propanol to determine hydroxyproline content. Likewise the presence of DMAB-reactive substances has been used to provide a tissue measure of integrated hyperglycaemia over prolonged periods of time in streptozotocin-diabetic rats[68].

DMAB has also been used as a reagent in studying many enzyme activities especially those related to the semi-synthesis of antibiotics. The soluble penicillin G acylase (PGA) from *Bacillus megaterium* was used for the synthesis of cefaclor. The enzyme activity was determined by a spectrophotometric assay with DMAB as a colorimetric substrate[69].

Simple and sensitive spectrophotometric and radiochemical procedures were described for the assay of acetyl-CoA: arylamine N-acetyltransferase (NAT: EC 2.3.1.5) which catalyzes the reaction acetyl CoA + arylamine - N-acetylated arylamine + CoASH. The methods were applicable to crude tissue homogenates and blood lysates[70].

The effective production of 7-aminocephalosporanic acid (7-ACA) is a matter of concern in the pharmaceutical industry because it is a starting material for the synthesis of semi synthetic cephalosporin. Therefore screening for new source of cephalosporin acylase positive bacteria is very important. The cephalosporin acylase can be found in several *Pseudomonas* sp. and other bacteria. To facilitate the attempts of obtaining the microorganisms with higher cephalosporin acylase activity from natural environments, development of new and specific methods for screening environmental microorganisms with

cephalosporin acylase activity is very important. For detection of microorganisms with cephalosporin acylase activity, bacteria were grown for 3 day; then each colony on the plate was exposed to chloroform vapour for 15 min, scraped with a toothpick, and suspended in 100 µL of GL-7ACA (1 mg of GL-7ACA or cephalosporin C per ml of 0.1 M phosphate buffer [pH 7.0]) in a well of a microtitre dish. The mixture was incubated at 37°C for 60 min, and the reaction was terminated by addition of 120 µL of acetic acid : 4.25 M NaOH (2:1), followed by addition of 40 µL of *p*-dimethylaminobenzaldehyde (0.5% in methanol). *Para*-dimethylaminobenzaldehyde forms a yellow condensation product with 7-ACA or cephalosporin C[71].

A grapelike odour is often of diagnostic importance in detecting the growth of *Pseudomonas aeruginosa* in culture and in burn wounds. The compound responsible for the odour has been identified as 2-aminoacetophenone (2AA) by mass spectroscopy. Although the grape odour is sometimes difficult to detect in culture media, gas chromatographic, fluorimetric, and colorimetric methods can be utilized to assay 2-aminoacetophenone production in a variety of media. Its synthesis occurs relatively early in the growth cycle. It has proved easy and convenient to detect 2-aminoacetophenone excretion by *P. aeruginosa* after 24 h of incubation on blood agar plates employing a fluorimetric assay of ether extracts of the agar medium. Thin-layer chromatograms of authentic 2AA and ether extracts of alkaline culture media yielded single, yellow spots at the position of 2AA when the developed chromatograms were sprayed with Ehrlich reagent (1 g of *p*-dimethylaminobenzaldehyde in a solution containing 25 mL of HCl and 75 mL of methanol)[72].

In order to study enzyme immobilization on chitosan activated with glutaraldehyde, aiming to produce a cheap biocatalyst, two different immobilization strategies were studied: one-point and multipoint covalent attachment to the solid matrix. The multipoint covalent attachment derivative had an 82% immobilization yield. Enzyme activity was assessed *via* colorimetric analysis using DMAB[73].

The various factors and conditions that can lead to production of mutant strains of microorganisms have also been studied by adopting DMAB as a reagent for monitoring one metabolite or another. Two mutants have been described in which the synthesis of tryptophanase is unusually insensitive to catabolite repression. Transductants were purified and grown overnight in L-broth. The addition of DMAB showed whether or not indole (made by Tna+ but not Tna- strains) was present[74].

The chemical modification of penicillin G acylase (PGA) obtained from a mutant of *Escherichia coli* ATCC 11105 was studied in order to identify the catalytically essential amino acid residues of the enzyme. The modification of PGA by serine specific phenylmethylsulphonylfluoride (PMSF) and tryptophan specific *N*-bromosuccinimide (NBS) resulted in the complete inactivation of the enzyme. DMAB method was used for kinetic investigations instead of the hydroxyl amine method[75].

The effects of the I5 polyol compounds on the thermostability of penicillin G acylase (PGA) from a mutant of *Escherichia coli* ATCC 11105 were investigated by monitoring acylase activity with DMAB[76].

The effect of pHs between 2.0 and 10.0 on the inactivation kinetics of penicillin G acylase (PGA) obtained from a mutant of *Escherichia coli* A TCC 11105 and the stabilization of enzyme against pH by chemical cross-linking with dimethyladipimidate (DMA) were studied with enzyme activity monitored with DMAB method[77].

The induction of ergot alkaloid synthesis at enzymatic level was studied by measuring the level of tryptophan using the DMAB method[78].

3.3 Some Biomedical Applications

DMAB has also been utilized for the biomedical analysis of endogenous substances. Diagnostic tests on 102 male patients suspected with cannabis abuse were done. Liquid-liquid extraction of cannabinoids from urine was done and screened by Duquenois-Levine, fast blue B salt and p-dimethylaminobenzaldehyde (p-DMAB) tests. All the results were confirmed by high performance thin layer chromatography (HPTLC). Samples were considered positive for cannabis based on the positive indication in colour test and by detection of 11-nor-Δ^9 tetrahydrocannabinol-9-carboxylic acid (THC-COOH) on HPTLC [79]. Likewise, *p*-amino compounds in acidic and ethanolic solution combine directly with DMAB to yield a yellow product. The color formation is pH sensitive, with an optimal pH zone. This has been used for analyzing biological fluids for primary arylamines and especially for the determination of *p*-aminohippuric acid in renal function studies[80]. This method is a modification of previously developed method for sulphanilamide[81] and *p*-aminohuppuric acid[82] in clinical samples.

3.4 Synthetic Applications

DMAB has found a profound use in many synthetic designs as a suitable carbonyl donor and for the introduction of *para*-dimethylaminobenzene substituents into many synthetic compounds. The formation of most reported Schiff bases- imines, hydrazones and semicarbazones have all adopted DMAB as one of the aldehyde donors. This section of this paper reviews some of the synthetic applications where DMAB has found relevance in their designs.

3.4.1 Schiff bases

Perhaps the major synthetic application of DMAB has been in the formation of varied types of Schiff bases. Schiff bases are formed by the condensation of a carbonyl group with an amino donor. The general synthetic pathway for these compounds is presented in Figure 8.

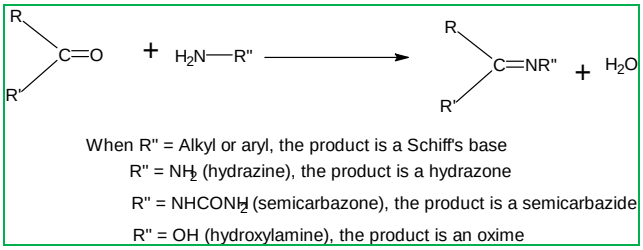


Figure 8: General Synthetic Pathway for Schiff bases and related compounds

A 1-(4-dimethylaminobenzyl)-2-(4-dimethylaminophenyl)-benzimidazole was synthesized by the reaction of DMAB and *o*-phenylenediamine. The structure was established by X-ray crystallography and found not to be the regularly reported rearrangement product, N, N`-bis(4-dimethylaminobezylidene)-benzene-1,2-diamine[83].

Complexes of Iron, Cobalt, Nickel and Zinc ions with the Schiff base derived from DMAB and *o*-aminobenzoic acid were synthesized and investigated by several techniques using elemental analysis (C,H,N), molar conductance measurements, infrared and electronic spectra. The elemental analysis data suggest the stoichiometry to be 1:1 [M:L] ratio formation[84].

Similarly, a novel N-Substituted-phenyl-1,2,3- triazole-4-acylhydrazone was prepared by the condensation of DMAB with 1-Phenyl-1H-1,2,3-triazole-4-carbohydrazide. The new compound was found to have profound anti-platelet aggregation properties[85].

DMAB has also been used for the synthesis of thermotropic substances. In one of such synthesis, a new series of Schiff base esters, 4-(dimethylamino)benzylidene-40-alkanoyloxyanilines containing even number of carbons at the end group of the molecules ($C_{n-1}H_{2n-1}COO$, n = 6, 8, 10, 12, 14, 16, 18) were synthesized. The compounds were monotropic liquid crystals. It was also found that the end groups of the molecules had effect on the mesomorphic properties[86].

In another application, a series of neutral bis-ligand Cu^{II}, Ni^{II}, Pd^{II} and Pt^{II} chelates with Schiff base ligands derived from S-benzyldithiocarbazate and DMAB were prepared and characterized. The Schiff base acts as a single negatively charged bidentate ligand forming stable neutral metal complexes. Magnetic and spectroscopic data suggest a square-planar structure for the Ni^{II}, Pd^{II} and Pt^{II} chelates. Also, ESR spectral and variable temperature magnetic susceptibility data support the square-planar structure of Cu^{II} chelate[87].

Arulmurugan et al recently presented a comprehensive review of majority of Schiff bases and their metal complexes synthesised, characterised both physicochemically and pharmacologically. Majority of these Schiff bases are produced using DMAB with other amino donors[88].

Aldehyde imines, the simplest of all Schiff bases, are not stable at room temperature, undergoing condensation and polymerization reactions. However, in an application of DMAB, dimethylaminobenzaldimine–zinc-bis (pentafluorothiophenolate) was obtained from the reaction of DNAB with ammonia-contaminated zinc-bis(pentafluorothiophenolate) and characterized by a structure determination. Zinc effects the catalytic formation and stabilization of the aldimine by complexation[89]. The structures and molecular projections of the compounds are presented in Figure 9.

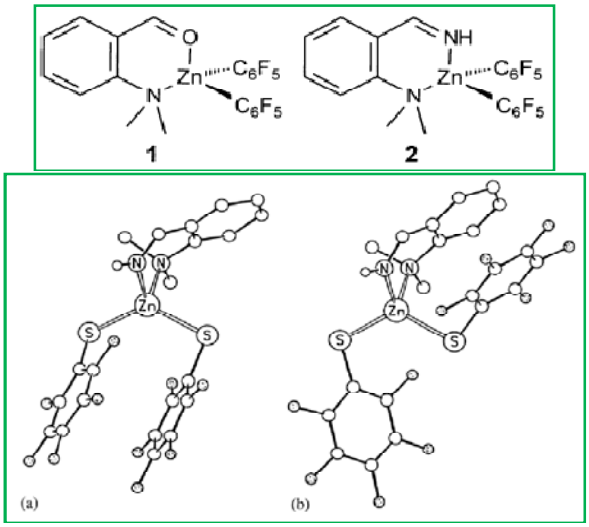


Figure 9: Structures and molecular projections of Zinc-Aldimine, (a): ring-parallel conformers in 2a, 2b and 2c(1). (b): ring-separated conformer in 2c(2) (ref 89).

3.4.2 Semicarbazones

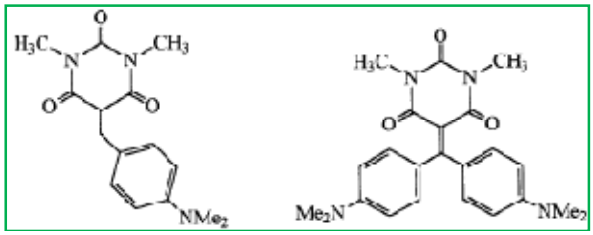
Second-order nonlinear optical (NLO) materials have been attracting considerable attention due to their broad applications in optoelectronics, such as optical frequency conversion and optical parameter oscillator. It has long been recognized that molecular chromophores could exhibit a second-order NLO response several orders of magnitude larger than that of inorganic compounds, such as potassium dihydrogen phosphate and lithium niobate. In this regard, DMAB thiosemicarbazone was synthesised alongside others with DMAB thiosemicarbazone showing the largest first-order molecular hyperpolarizabilities (β)[90].

Similarly, a new series of 2-[(phenylmethylene) hydrazono]-4-oxo-3-phenyl-5 thiazolidineacetic acids were synthesized. Benzaldehyde 4-phenyl-3-thiosemicarbazones substituted (including DMAB) were also obtained and used as intermediate to give the title compounds. All synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR. The *in vitro* anti-*Toxoplasma gondii* activities of the compounds were evaluated with IC_{50} ranging from 0.05-1nM[91].

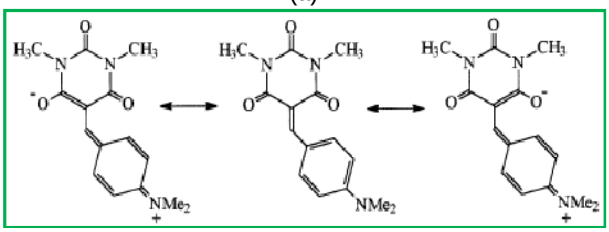
3.4.3 Dye Synthesis

DMAB has also found application in the synthesis of various dyes with different possible applications. The pyrimidinetrione system of barbituric acid may be envisaged as a potentially useful fragment in the design of new cyanine dyes. Conjugation of the carbonyl groups of

this ring system with a nitrogen or oxygen atom of another fragment by means of a polymethine chain should lead to new dyes with interesting spectroscopic properties. The preparation and the solvatochromic behaviour of two dyes, obtained by condensation of N,N`-dimethylbarbituric acid with DMAB and with 4,4`-bis(*N*,*N*-dimethylamino)benzophenone (Michler's ketone) have been described[92]. The structures of the dyes and their ionic forms in protic solvents are presented in Figure 10.



(a)



(b)

Figure 10: Merocyanine-type dyes from barbituric acid derivatives (a)-the two dyes and (b)-various ionic forms in protic solvents (ref 92).

The synthesis and characteristic absorption spectra of 2,3,8-trisubstituted-indeno[2,1 b]thiophene compounds have been reported. Cyclization of 3-dicyanovinyl-indan-1-one with sulphur gave 2-amino-8-oxo-8H-indeno[2,1-b]thiophene-3-carbo-nitrile compound. The reaction of the latter with malononitrile and with ethyl cyanoacetate gave the indeno[2,1-b]thiophene derivatives. The absorption maxima of these compounds changed very significantly by the introduction of various substituents on the 8-position of indeno[2,1-*b*]thiophenes ring system. The preparations of the corresponding azomethine dyes and azo disperse dyes were also reported. Three azomethine dyes were prepared by condensation reaction of nitrile and thiophenes derivatives with DMAB[93].

Cyclic 1,3-diones like Meldrum's (2,2,-dimethyl-1,3-dioxane-4,6-dione) and barbituric acid may be used as building blocks for intramolecular donor– acceptor pairs which act as solvatochromic dyes. Thus, condensation of DMAB with N,N-dimethylbarbituric acid leads to the formation of merocyanine. In this regard, The preparation of the 5-(4-N,N-dimethylaminobenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione, a dye derived from Meldrum's acid, was described, and its solvatochromic behaviour compared with that of an analogous derivative of barbituric acid [94]. The dye was particularly found to be sensitive both to the dipolarity–polarizability and the acidity of the medium.

Cyanines are a class of dyes whose chemical structure is characterized by two nitrogen atoms (one of which is positively charged), which are separated by a conjugated bridge formed by a carbon framework. The importance of these dyes stems from their wide use in industries for many years as spectral sensitizers for silver halide photography, in optical disks as recording media, as photorefractive materials, in laser devices and even as anti-tumour reagents. One of the cyanine dyes was prepared by coupling bromooctanoic acid with γ-picoline, which was then condensed with DMAB to afford the desired product as red solid [95].

A series of novel 4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno[2,3-d]pyrimidinium and 5-oxo-1,2,3,5-tetrahydropyrrolo[2,1-b]quinazoliniumstyryl dyes were synthesized. For preparing of studied dyes the standard method of styrylcyanines synthesis was modified. Spectral-luminescent properties of obtained dyes in the free state and in the presence of nucleic acids and BSA were studied. It was shown that *p*-dimethylaminostyryls based on 4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]thieno [2,3-*d*]pyrimidinium with aliphatic substituents in 2 and 3 positions demonstrated RNA binding preference. These dyes in the presence of RNA significantly enhance emission intensity and could be used as RNA-specific fluorescent probes. Besides, the fluorescence emission after two-photon absorption of dye-RNA complexes in buffer solutions was measured[96]. One of the dyes was formed by a condensation reaction with DMAB.

3.4.4. Miscellaneous Synthetic Applications

Para-dimethylaminobenzaldehyde has found some other useful synthetic applications which do not fall under the categorization stated in the paragraphs above. Thus, this section reviews some other important synthetic applications of DMAB.

Azulene and its derivatives constitute a highly interesting class of compounds due to the fused 5–7 bicyclic aromatic ring system. These compounds are regarded as one of the representative examples of non-benzenoid aromatic hydrocarbons, which were found to be reluctant to undergo Diels–Alder reactions but easily susceptible to various electrophilic substitution reactions such as acylation, halogenation, nitration, azo-coupling and aminomethylation most easily at the C-1 and/or C-3 position. In particular, naturally occurring guaiazulene (1: 1,4-dimethyl-7-isopropylazulene) possesses low oxidation potential (E^{ox} +0.65 V vs SCE) in comparison with those of azulene (E^{ox}+0.88 V vs SCE) and other alkylazulenes (E^{ox} +0.88– +0.67 V vs SCE), and is an interesting compound from the viewpoint of the creation of novel functional materials, with a delocalized p-electron system possessing a 3-guaiazulenyl group (Gu^3), which serves as an electron donor. Thus, reaction of guaiazulene with DMAB in methanol in the presence of tetrafluoroboric acid gives the title mono-carbocation compound, [4-(dimethylamino) phenyl] -3-guaiazulenyl methylium tetrafluoro borate, in 90% yield [97].

Search for new materials with high optical nonlinearities has been the important task because of their practical application in harmonic generation, amplitude and phase modulation, switching and other signal processing devices. In this regard, an organic nonlinear optical material, 4-chloro-4-dimethylamino-benzylidene aniline (CDMABA), was synthesized by the condensation of the *p*-chloroaniline and *p*-dimethylaminobenzaldehyde. Solubility of CDMABA was determined in acetone at different temperatures. Single crystals were grown by the solvent evaporation method from acetone solution at room temperature. Grown crystal was subjected to FTIR, FTRaman and ^{1}H NMR spectral analyses to confirm the synthesized compound. The range and percentage of optical transmission was ascertained by recording UV–Vis–NIR spectrum[98].

A novel and simple fluorophore, *p*-dimethylaminobenzaldehyde thiosemicarbazone (DMABTS), was prepared in order to find available fluorescent chemosensor for mercuric ion in aqueous solution. DMABTS emitted fluorescence at 448 nm in aqueous solution and its fluorescence intensity was completely quenched upon interaction with Hg^{2+} ions, which should be attributed to the 1:1 complex formation between DMABTS and Hg^{2+}. The coexistence of several transition metal ions and anions did interfere with the fluorimetric titration of Hg^{2+} ion by less than 4 % in the emission change[99].

Hybrid mixed metal oxalates of general formula $[M_2^{III}M^{II}(C_2O_4)_6][DAMS]_4.2DAMBA.\ 2H_2O$, where M^{III} = Rh, Fe, Cr; M^{II} = Mn, Zn; DAMBA = *para*-dimethylamino benzaldehyde and [$DAMS^+$] = trans-4-(4- dimethylamino styryl)-1-methylpyridinium, belong to a new family of multifunctional materials displaying both very high second harmonic generation (SHG) efficiency and tunable magnetic properties. Cariati et al reported the preparation and magnetic characterization of the new Ni^{II} members of this family [100].

DMAB has also found practical relevance in the cross-coupling reaction of arylbromides and chlorides[101], reductive C-alkylation of barbituric acid derivatives in the presence of palladium and platinum catalysts[102] as well as in the reductive mono-alkylation of nitroaryls[103].

Oniţa et al recently reported the synthesis of Aminophosphonates as potential herbicides for which DMAB served as one of the precursors. The antioxidant activity was tested in experimental assays with DPPH• (1,1-diphenyl-2-picryl-hydrazyl) and the potential to function as herbicides, in red blood cells membrane destruction experiments. Regarding the reaction with DPPH•, at least 3 aminophosphonates are better antioxidants than 2,6-di-tert-butyl-4-methylphenol (BHT). Aminophosphonates' capacity to function as herbicide (demonstrated through their haemolytic activity) is given by the presence in their structure of some shorter aliphatic substituents and / or more benzene nuclei[104].

4.0 MISCELLANEOUS APPLICATIONS

This part of this review reports some other applications where DMAB has been utilized either as a synthetic precursor or reagent for analysis. The formation of an alcohol from the reaction of DMAB with pyrrole derivatives having intact CH-group in the α or β position relative to the cyclic NH-group have been reported and DMAB reagent was utilised for the spectrophotometric determination of 2-phenylindole by Gillio-Tos *et al*[105]. In a similar procedure, Adegoke and Osoye[106] found that reactive methylene centres generated by artemisinin derivatives (artesunate and dihydroartemisinin) *in situ* in an acidic medium produced a purple-coloured solution which was used for the full colorimetric determination of these important antimalarial agents.

DMAB has also been used for the histochemical demonstration of tryptophan and related compounds[107].

5.0 OVERVIEW OF THE UTILITY STATUS AND FUTURE PROSPECTS

This paper has made attempt to review the many diverse applications of DMAB which spanned over a century. DMAB because of its peculiar structural features has found applications in synthetic, biochemical, biomedical and analytical sciences. Its use in these respects is gradually increasing and some of the age-long applications of this reagent are experiencing some new modifications. The relevance of this compound in microbiology and analytical chemistry has been unparalled by any other reagent in history. It behoves to opine that the relative lack of toxicity of this compound has made it more useful in these fields. However, the synthesis of DMAB appears unnecessarily too lengthy and one believes that the challenge for synthetic chemists will be to devise some simpler pathways for its synthesis. The future prospect of this reagent is still bright as its unique properties of being oxidizable and reducible will lend it to more usefulness. When a need for a dimethylamino substituted benzene molecule is arises DMAB comes handy and useful. Its ability to form condensation products readily with a wide range of chemical groupings such as amines, carboxylates and other aldehydes will make it more relevant in synthetic design and processes. Its recent application as a coupling component in our laboratory for the spectrophotometric determination of diazotized nitroimidazoles has further opened up another area of research into its utilization for which ready applications in the third world economies will be possible. The reaction also has a potential of being used as a pre-column derivatization procedure and for which analysis of pharmaceuticals in dosage forms and biological fluids will be possible.

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