INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

CHEMISTRY OF FORMAZAN

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ABSTRACT

Formazans was first synthesized over a century ago, but still attract attention of chemists, biologists, technologists and other specialists. In recent years, antiviral, anti-inflammatory, anti-fertility, anti-tubercular activity, antimicrobial activities, anti-cancer and anti-corrosion properties of formazans have been published. This review aims to describe the structures, synthesis, reactions and spectral properties of formazans for highlighting the future applications in several bioactive phenomena and analytical uses.

Keywords: Formazan, Tetrazolium salt, Coupling reaction, Phase transfer catalyst.

1. INTRODUCTION

The history of the tetrazolium salts and formazans goes back more than one hundred years, when Friese (1875) reacted benzene diazonium nitrate with nitro methane, to produce a cherry-red "Neue Verbindung". This was the first formazan(Altman, 1976). Nineteen years later, Von Pechmann and Runge oxidized a formazan to produce the first tetrazolium salt. Many hundreds of tetrazolium salts and formazans were prepared in the following years(Pechmann and Runge, 1894).

Formazans are compounds which contain the characteristic chain of atoms (N=N–C=N–NH), formazans arecoulored compounds ranging from red to orange or blue depending upon their structures.

Most reports on formazans originate in the early 1900s, preceding modern characterization techniques. As a result, there is relatively little known about their properties. However, the common feature of all formazans is their intense colour, which allowed chemists to identify them over 100 years ago(Gilroy, 2008).

Redox behaviors of formazans were evaluated in detail. Formazans form tetrazolium salt when they are oxidized. Tetrazolium salts are reduced back to formazans by the enzymes in the cell and stain the tissue. Tetrazolium–formazan system is classified as a marker of vitality. This feature enabled thedetermination of activity on tumor cell, which caused an increasing interest in the chemistry, and especially electrochemistry of formazans(Tezcan *et al.*, 2008).

Formazans have been studied extensively because of their ready accessibility, diverse chemical reactivity, broad spectrum of biological activities and wide range of applications(Chavan*et al.*, 2012, Anand*et al.*, 2009, Lipounova*et al.*, 2004).

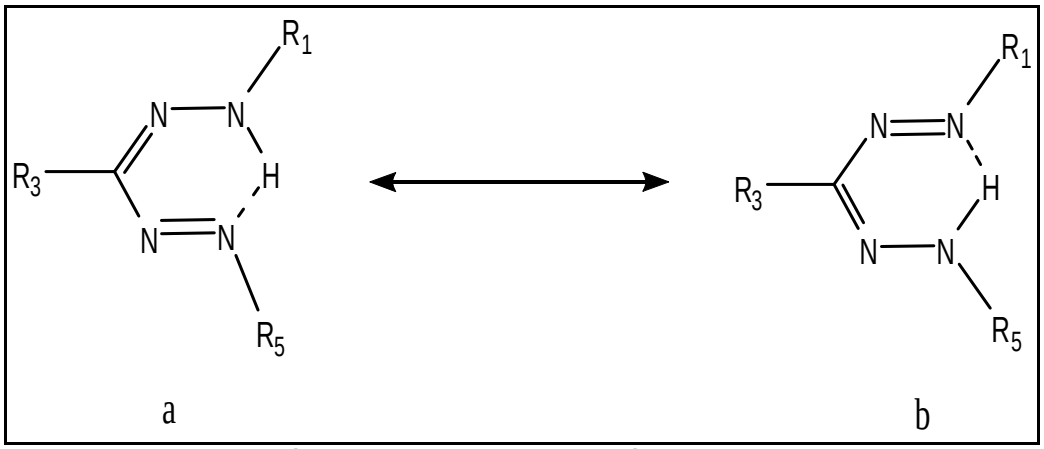
They are widely used as dyes, as ligands in complex formation reactions, and as analytical reagents, where their deep colour makes them good indicators of redox reactions. Formazans form salts and complexes with several metal ions, and especially the transition metal ions. Their derivatives with electron donating and withdrawing group attached to 1,3,5-phenyl ring were synthesized and the effects of substituents on the absorption λmax values were examined. They are also biologically active and there is much interest in their biological applications. The biological activity of formazan makes the knowledge of its oxidation potentials and possible mechanisms very important(Tunça and Yıldırım, 2010).

2. Structures of Formazan

Formazans are characterized by intense coulors, ranging from cherry red to a deep purplish black and contain the characteristic chain of atoms -N=N-C=N-NH-. Formazans are generally solids of relatively low melting point in spite of large the size of the molecules. Triphenyl formazans are often particularly soluble in chloroform and acetone; in water the solubility appears to be negligible, the solvent being coulored.

Their structure was first revealed by Bamberger and by von Pechmann who agreed to call them formazyl compounds. In 1933, German usage is exemplified by Beilstein, in which the compound is termed formazan. The compound is substituted with three phenyl groups at (R, R', R") which is called 1,3,5-triphenyl formazan(Şenőz, 2012).

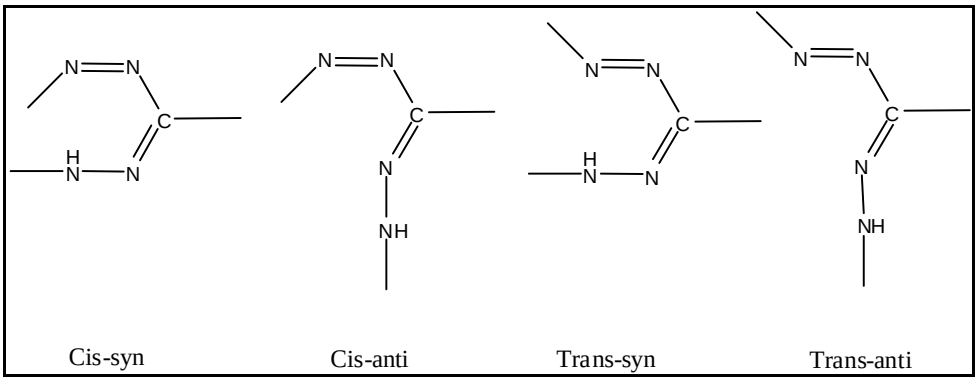
The structure of formazans was rather complex. The tautomerism of formazans, first described by Penchmann and Runge(Penchmann and Runge, 1894), but his results were inconclusive. In 1941(Hunter and Roberts, 1941), Hunter and Robertsconclusively established for several pairs of formazans that the individuals in each pair were identical, although previously were described as tautomeric. They suggested that formazans were resonance hybrids with a chelated hydrogen-bridge structure. These workers therefore proposed an internally coordinated hydrogen-bond structure, which can exist in two mesomeric forms, a and b, (Scheme 1). The formazan molecule thus appears to be a resonance hybrid of these forms.



Scheme 1: Formazan mesomeric structures

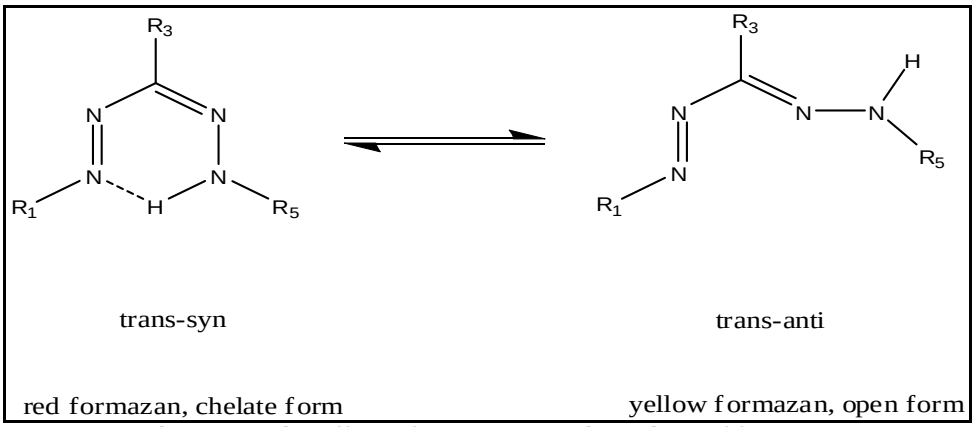
Triphenyl formazans exist exclusively as *pseudo* 6-membered rings in solution and the solid-state. However, other formazans with relatively small 3-substituents have been observed to adopt different structures(Gilroy, 2008).

Hausser *et al.*(Hausser *et al.*, 1949), showed that some formazans could be changed from red to yellow forms upon exposure to visible light. A formazan molecule allows the existence of four possible structures due to geometrical isomerism about the two double bonds (C=N, syn–anti and N=N, cis–trans), (Scheme 2). The orientation of substituents about the (N=N) bond is described using *cis/trans* nomenclature while the orientation about the (C=N) bond is described using *syn/anti* nomenclature. Finally, substitution of the (C-N) bond is described as *s-cis* or *s-trans.* the possibilities of tautomerism being ignored for the present.



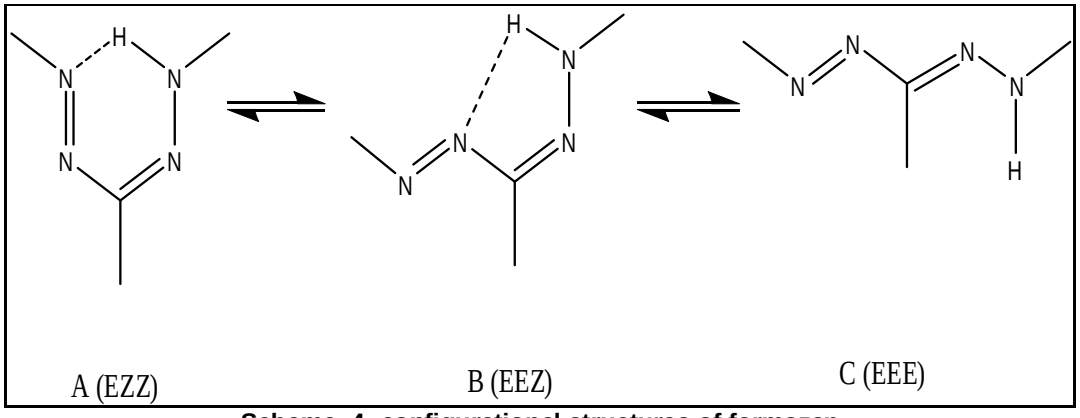
Scheme 2: Formazan geometrical isomers

As seen, cis-anti and trans-anti forms do not show chelate structure due to position of N-H, on the other hand cis-syn and trans-syn are observed to contain chelate structure involving hydrogen bonding. As the steric reason occurs in cis-syn, it is hardly possible to show chelate structure. Trans-syn form is the most favorable for the chelate structure. Formazan molecules involving hydrogen bridge are red while those which do not show chelate form are yellow (Şenőz, 2012), (Scheme3).
The presence of two multiple bonds in the formazan chain and the N5 atom with a lone pair electron brings about the formation of an overall conjugated system.



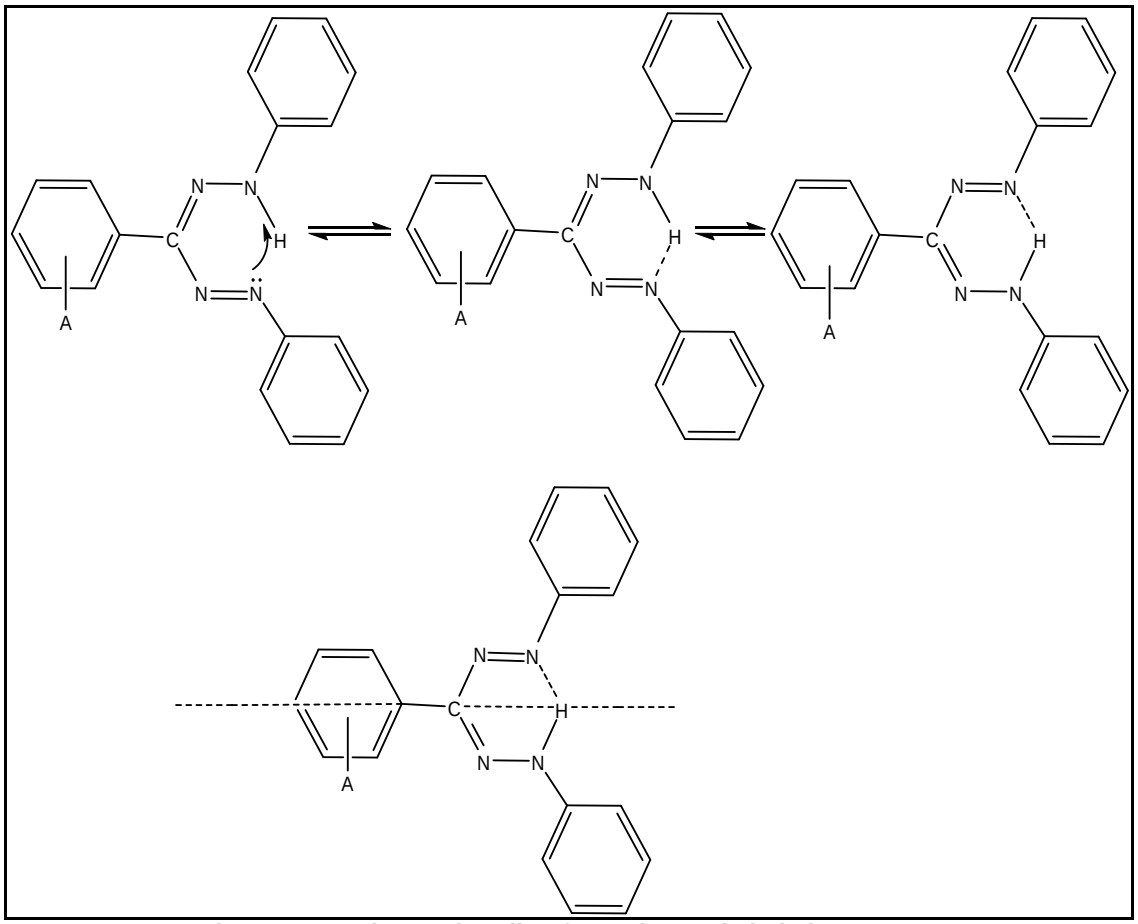
Scheme. 3: The effect of structure on the colour of formazan

Lipunova*et al.*(Lipunova*et al.*, 2006), has designated configuration of formazan structures in terms of E and Z instead of cis, trans, or syn, anti. X-Ray diffraction studies of a large series of formazans revealed three configurational combinations in the azohydrazone chains of the crystals, EZZ, EEZ and EEE. The configurations refer to the bond sequences N1–N2, N2–C3 and C3– N4, (Scheme 4).



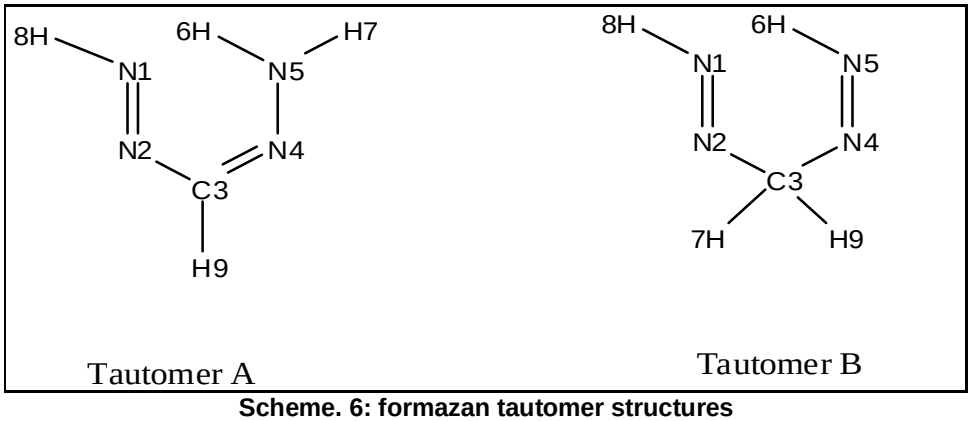
Scheme. 4: configurational structures of formazan

The configuration of formazans is largely determined by the steric effect of the substituent at the carbon atom. Thus EZZ isomers (structure A) are characteristic of formazans with a bulky substituent R_3(Ph, But, NO_2, etc.). In addition EZZ configuration is stabilized by a bridging N-H….N intramolecular hydrogen bond in the six-membered chelate ring. The results of ab initio quantum-chemical calculations of all possible conformations of these compounds suggest that the EZZ configuration is the most stable. The EEZ isomer is stabilized by the N_2-HN_5bond in the five-membered ring (structure B), while the EEE form is stabilized by intermolecular H-bonds. In N-azaheterocyclicformazans, which crystallize in the imino tautomeric form, the intramolecular N_4–HN (heterocycle) H-bond provides additional stabilization(Buemi*et al.*, 1998).
Tezcan and Özbek(Tezcan and Özbek, 2005), showed that NH hydrogen forms a hydrogen bond upon the electron pair on azo-nitrogen (-N=N¨-) and causing chelating and therefore tautomerism and there is a symmetry element in the molecule, (Scheme 5).



Scheme. 5: Hydrogen bonding formation and chelating property

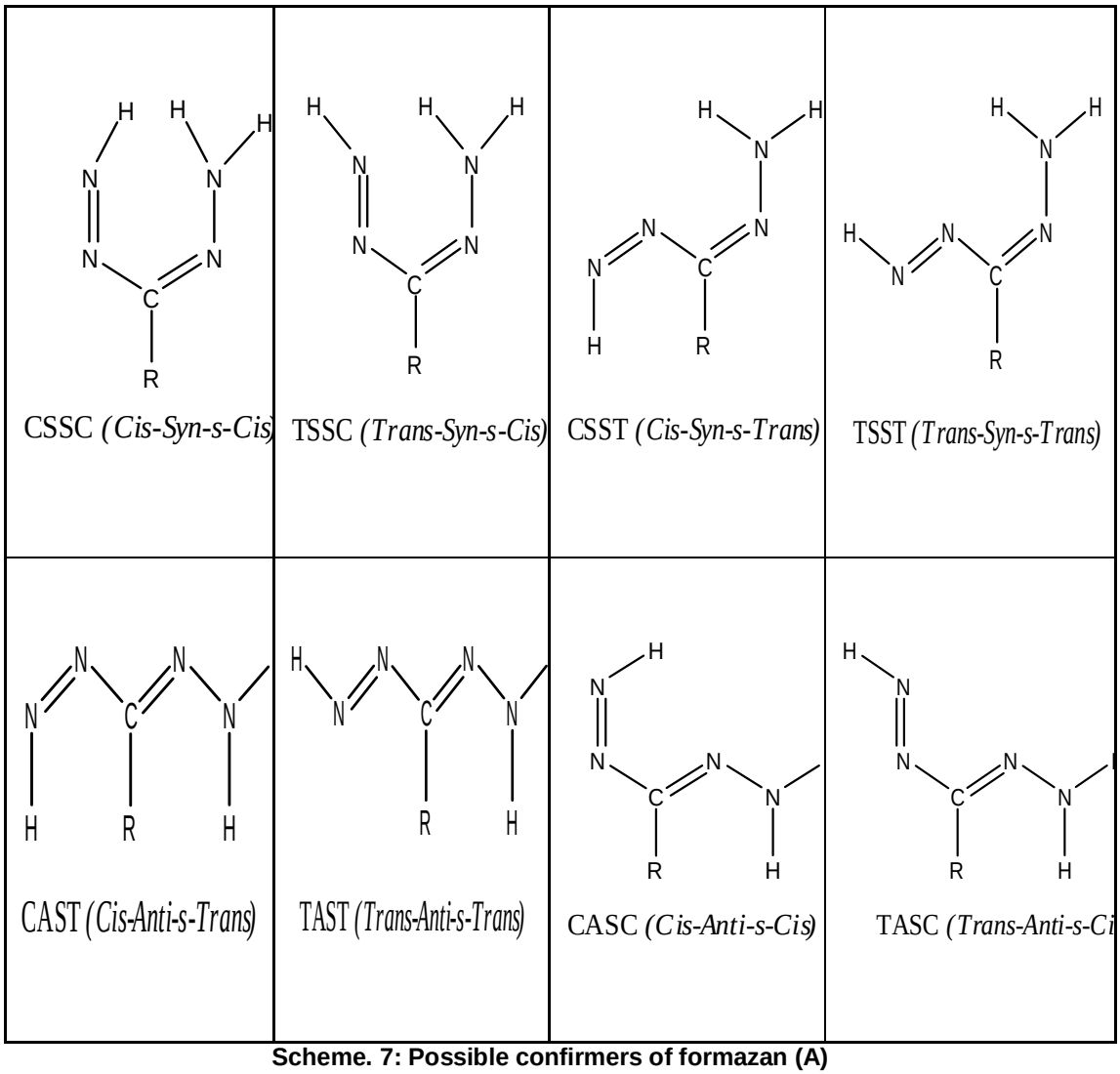
Buemi*et al.* (Buemi*et al.*, 1998), investigatedformazan and 3-nitroformazan at ab-initio level in all their possible conformations, for studying the various possibilities of intramolecular hydrogen bonding formation. The trans-syn-s-cis (TSSC), known also as yellow form, has been found to be the most stable conformer (at least in the gas phase) in both compounds. This particular structure is strongly stabilized by a(N-H..N)hydrogen bridge, which gives rise to a hexa atomic chelate ring, with the possibility of a proton transfer process. This closely resembles that of malondialdehyde, in the evolution of the potential energy shape but with a greater barrier height. Various approaches for obtaining a quantitativeestimate of the energy of the hydrogen bridges were discussed.



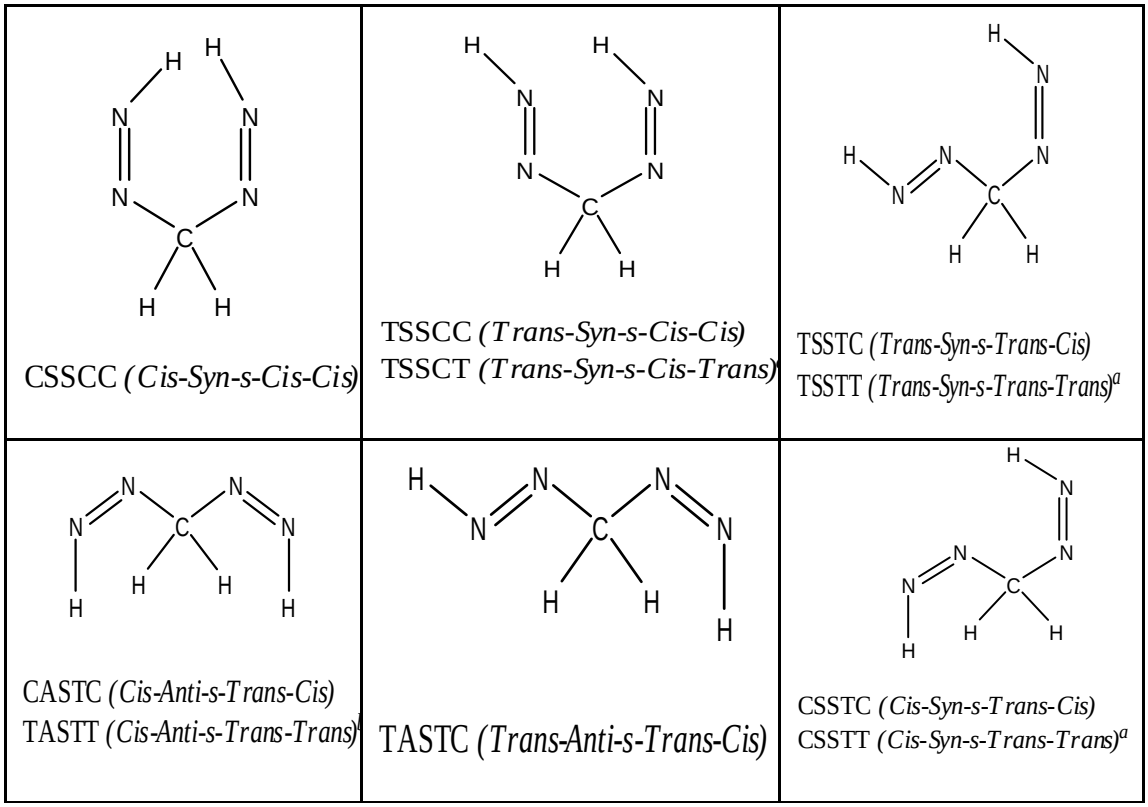
Scheme. 6: formazan tautomer structures

The electronic structures of the most favored TSSC, TSST (trans-syn-s-trans) and TAST (trans-anti-s-trans) conformations of formazan have been compared with those of the corresponding forms of 1,5-diphenylformazan, in order to account for the UV spectra available in the literature and the differentcoulors exhibited by the molecule on passing from one conformation to another. and they proved that the conformations of tautomer B are by far less stable than those of A (Scheme 6,7 and 8), mostly because conjugation and molecular planarity are broken so that the hydrogen bonding, no longer assisted by resonance, becomes very weak or disappears completely.

The stability of the formazan (electronic absorption stability, EAS) in low acid solutions can be explained by the influence of the substituents R_1, R_2 and $R_{2'}$ (Figure 1) on the electro-optical molecular parameters was revealed mainly at the level of the E_{LUMO} values which are consistent with much stronger stabilization of the ground electronic state in comparison to the excited state. The presence of the substituents resulted also in considerable increasing of the dipole moment and electron charge density values when compared to the formazan structure that have (R_1=R_2=$R_{2'}$=H) (Filip*et al.*, 2008).



Scheme. 7: Possible confirmers of formazan (A)



Scheme. 8: possible confirmers of formazan B.
[a] H6 rotated by 180°.[b] H6 and H8 rotated by 180°

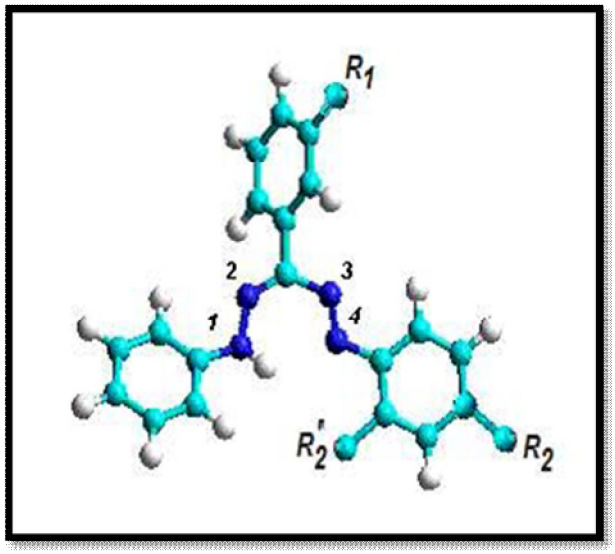


Fig. 1: Three dimensional structure of formazan(Filip*et al.*, 2008)

3. Spectral and Electronical Features of Formazan(Şenőz, 2012)

Formazans and their metal complexes are coulored compounds due to (– *) transitions of - electrons in formazan skeleton (–N=N–C=N–NH–) which caused intensive interest among the scientist.

Tezcan and Özbek(Tezcan and Özbek, 2005), and Tezcan *et al.* (Tezcan *et al.*, 2008, Tezcan *et al.*, 2013), synthesized formazan derivatives with electron donating and withdrawing group attached to 1,3,5-phenyl ring and examined the effects of substituents on the absorption *λ*max values.

Filip*et al.*(Filip*et al.*, 2009), revealed that The solvent effect on the electronic absorption spectra of some triphenyl formazan derivatives was revealed in the frame of this study. Binary solutions in different solvents were prepared, in order to reveal the nature of the solute-solvent interactions. The solute molecules can interact with solvent ones through orientation, induction and/or dispersion forces (universal interactions), as well as by specific interactions. All types of Van der Waals interactions depend on the microscopic parameters of the solute molecule, such as the dipole moment, polarizability, etc. The solvent effect can result in changes of the molecular energetic levels, which usually lead to electronic band shifts in the frequency scale, depending on the solvent macroscopic parameters like the refractive index and dielectric permittivity, and they found that the position and intensity of the UV band at 33,000 cm^{-1} (in ethanol) do not change in the protonation process, supporting the presumption that it can be assigned to - *) transitions. Small aliquots of sulfuric acid were added with the goal of UV band assessment either to (*n* - *) or to - *) transitions.

The spectra of triphenyl formazans exhibit four distinctive absorption bants (A,B,C, and D), one in the visible and the others in the UV range. The first band (A) observed in the wavelength range of 216-239 nm is assigned to that of the phenyl moiety. The second band (B, 240-285 nm) is attributed to the low energy - *)transition of the phenyl moiety. The third band (C) within the 300- 350 nm range and the sharp peak is due to the - *)transition within the hydrogen chelate ring formed by the azo and hydrazone group and the tautomerization occurring within this ring. The fourth broad band is characteristic of the formazan structure due to - *)transitions within the (N=N) group influenced by charge transfer within the whole molecule. The band is generally observed at 410-500 nm and shifted to 600 nm depending upon the structure. The visible absorption is very intensive, with the extinction coefficients registering values between 13.000- 23.000 for mono-formazans and 35.000-50.000 for di-formazans.

There are notable absorption bands in the IR spectra of formazans. These are (C=N), (N-H) and (N=N) absorption bands. Shifting toward lower or higher frequency of these bands determine chelate or non-chelate structure. The (C=N) stretching band at 1500-1510 cm^{-1}shows chelate structures. On the contrary, the (C=N) stretching band at 1551-1561 cm^{-1}shows non-chelate structures. The shifts of these band to higher frequencies are explained in terms of the rupture of hydrogen bond and the loss of the resonance stabilization of the six-membered chelate ring. Similarly, the lower values of the (N=N) stretching band are due to the intramolecular hydrogen bond and to some chelate ring resonance. The (N=N) stretching band of the chelate form of TPF is located at 1357 cm-1 while non-chelate form is located at 1418 cm^{-1}. In addition, the lower values of absorption bands (N-H) 3011-3090 cm^{-1}, showed chelate structure.

The majority of formazans with this form are generally characterized by the lack of (N-H) absorption band. Chelate structures have a six-membered conjugated system that p-electrons are delocalized. Because of this, double bond characterize decreases. And the stretching bands of (C=N), (N=N) and (N-H) were observed at lower frequencies.

The (N-H) signal of formazan in the NMR spectrum is indicative in evaluating the structure. (N-H) signal in the downfield region at δ 16 exhibits intramolecular hydrogen bonding, while upfield shifts of this signal at δ 10 indicate a weakening of the intramolecular hydrogen bonding.

Umemoto (Umemoto, 1985), claimed that ditetrazolium salts are reduced to both mono and diformazans by one electron transfers. The first 1 electron transfer results in the formation of a tetrazolium radical. He claimed that the reaction key leading to the simultaneous generation for mono and diformazans is the disproportionation reaction. It was also claimed that in polarographic study of formazans there were two irreversible diffusion controlled processes, each one with 1 electron transfers.

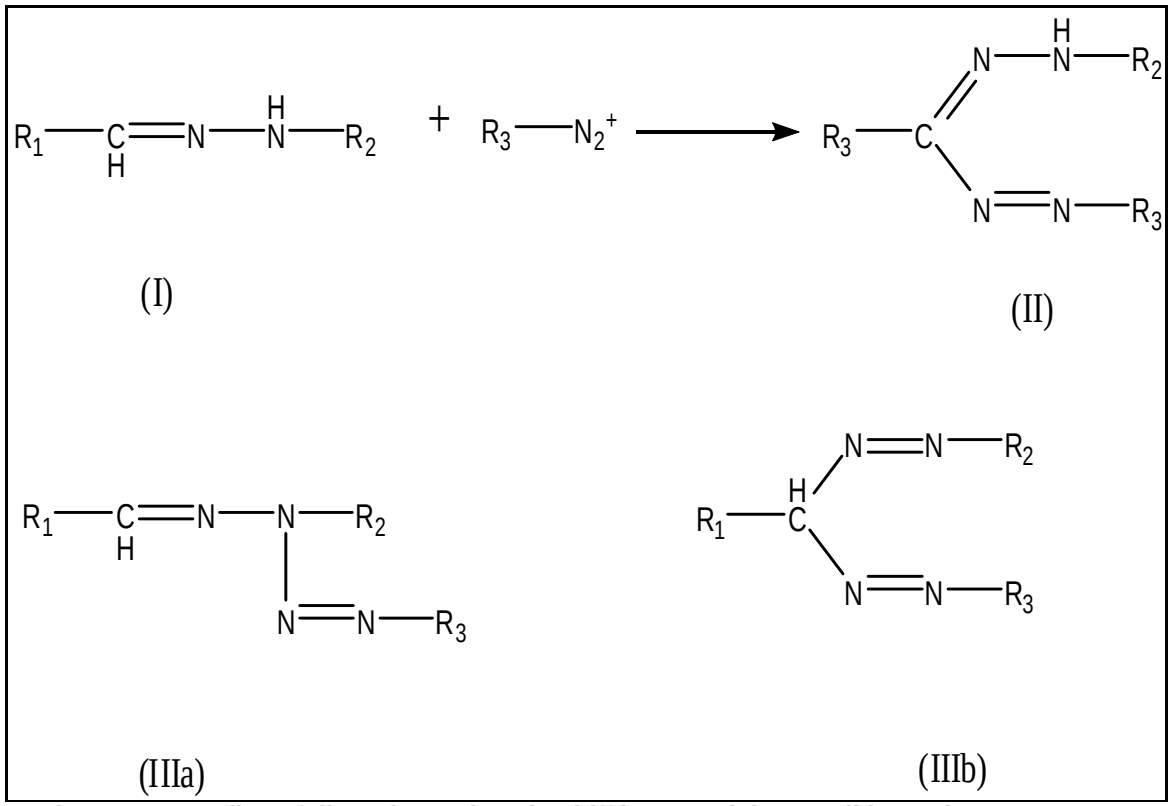
Abu Elenien(Abu Elenien, 1994), reported that formazans are oxidized in a single 2 electron transfer followed by a deprotonation reaction forming corresponding tetrazolium cation. In a study of the reduction of tetrazolium salts into formazans with superoxide ions, claimed to be the cause of aging and various diseases in human body, there was 1 electron transfer at −0.20V (Ag/AgCl) and one $1e^{-}/1H^{+}$ transfer at −0.40V (Ag/AgCl) (Oritani*et al.*, 2004).

4. Preparation Methods of Formazan

4.1. Coupling of Diazonium Salt With SchiffBases (Hydrazones)

The reaction of diazonium compounds with Schiff base (I) leads to the formazans (II). By carrying out the reaction under carefully controlled conditions at pH 6-8(Tezcan *et al.*, 2008)**.**Busch and Schmidt(Busch and Schmidt, 1931),isolated a light yellow intermediate formulated as the tetrazene (IIIa), (Scheme9). This intermediate was shown to isomerise to the deep red formazan even in the solid state and very rapidly in basic solution. However,Hegarty and Scott proved that the intermediate had been not shown unambiguously to be the tetrazene (IIIa) and they presented evidence to show that this structure is incorrect, and the structure (IIIb) is correct. The rearrangement of the intermediate to the formazan thus, rather than involving an intramoleculartransfer of the diazo function from nitrogen to carbon, is merely an azo hydrazone conversion(Hegarty and Scott, 1966).

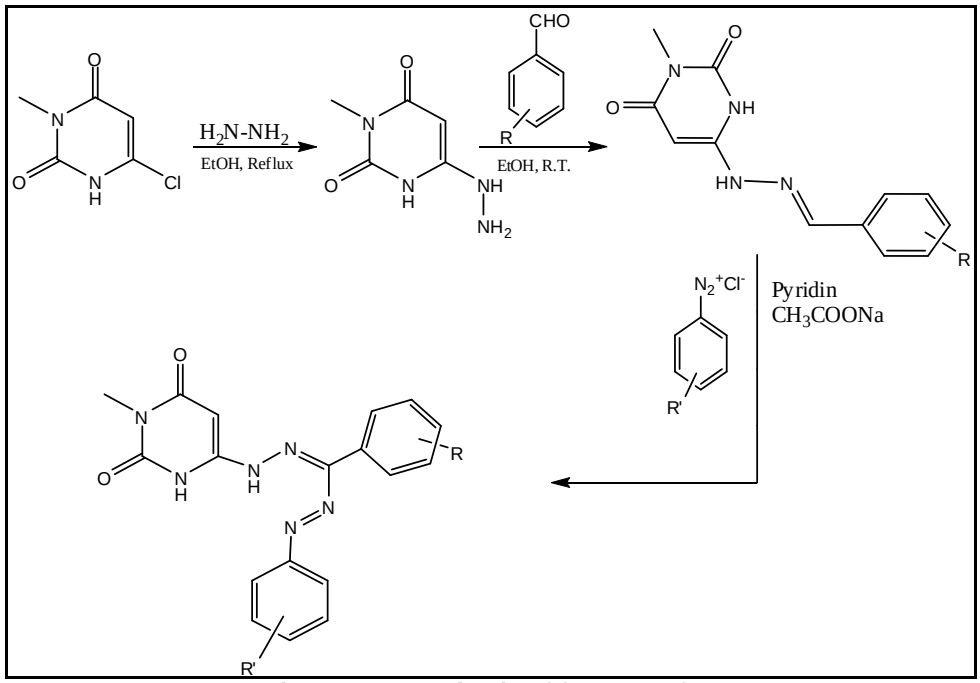
Tezcan and Özbek(Tezcan and Özbek, 2005), investigated the fact that (NH) proton is more acidic than (CH) proton, the first diazonium coupling realized through (NH) proton forming (IIIb), which is highly unstable intermediate and gives intramolecular rotation turning into formazan (II).



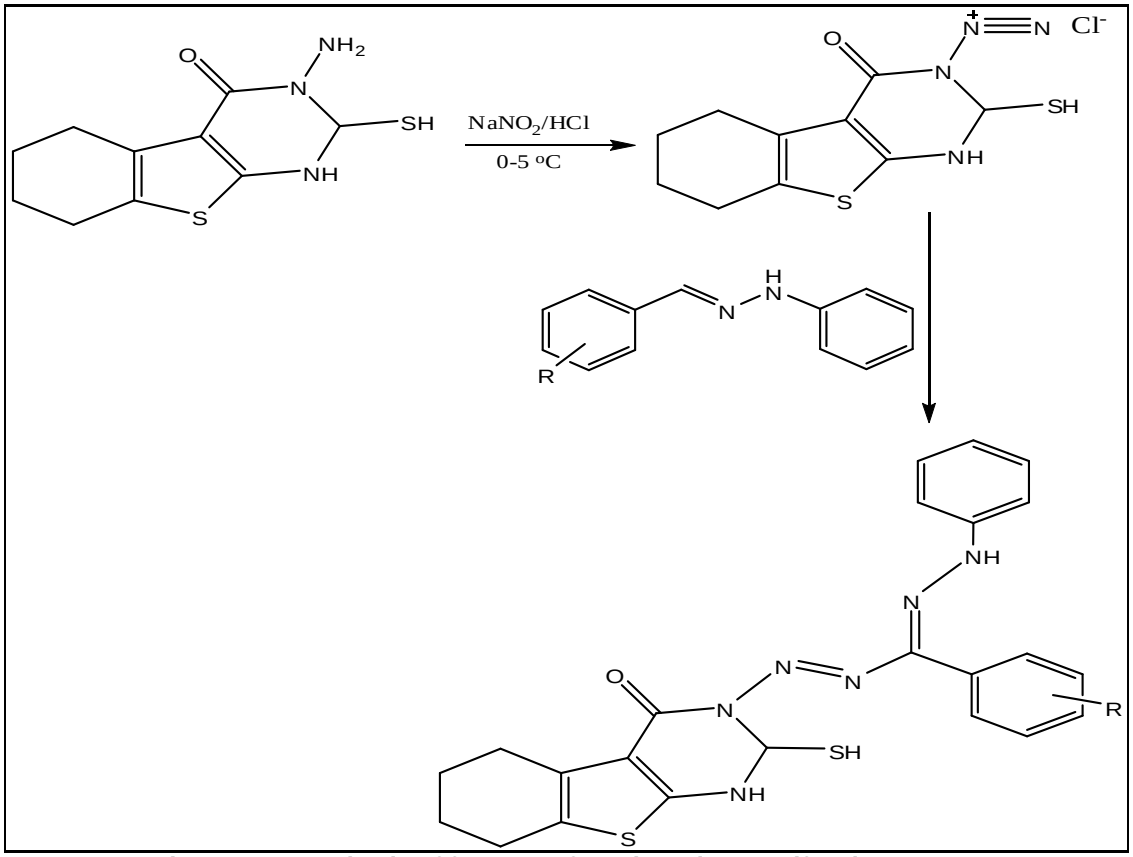
Scheme. 9: Coupling of diazonium salt and Schiff base, and the possible product structures

From here we start learning about preparation of triphenyl formazan by condensation of aromatic and aliphatic aldehydes with phenyl hydrazine and the coupling reaction of the resulting hydrazones with diazonium salts. The majority of known formazans are generated by this method, which is the standard one for the triphenyl formazans.

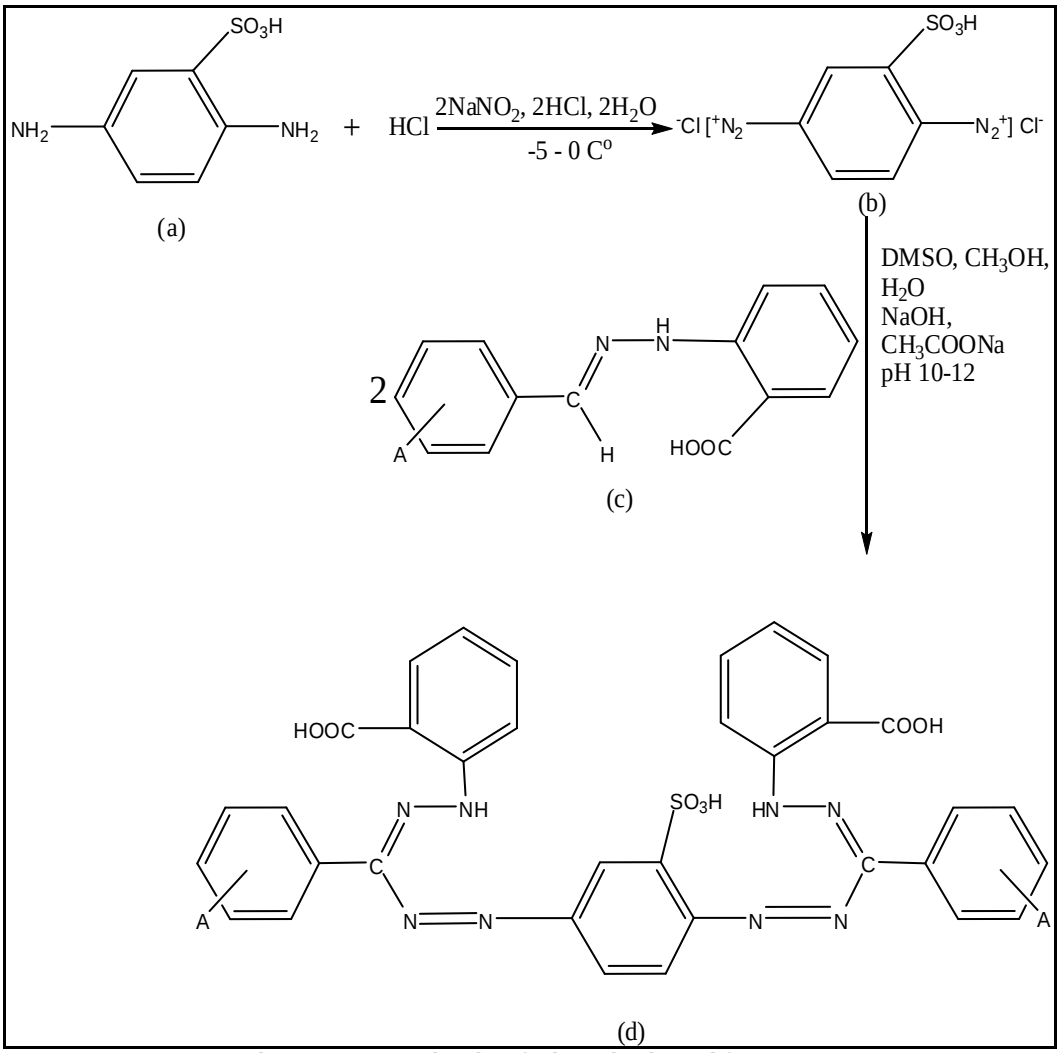
Not only substituted starting material, but also heterocyclic derivatives of aldehyde, hydrazine and amines were used to synthesize formazans. such as Samel and Pai(Samel and Pai, 2010), used heterocyclic hydrazine (6-hydrazino-3-methylpyrimidine-2,4-(1*H*,3*H*)-dione), (Scheme 10). Lakshmi*et al.* (Lakshmi*et al.*, 2009),used heterocyclic amine (3-amino-2-sulfanyl-2,3,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]pyrimidin-4(1*H*)-one), (Scheme 11).Bis-substituted formazan (d), also synthesized by coupling reaction of 2,5-diaminosulfonic acid (Tezcan and Özbek, 2005), (Scheme12).



Scheme. 10: Synthesis of formazan from (6-Hydrazino-3-methylpyrimidine-2,4-(1*H*,3*H*)-dione)



Scheme. 11: Synthesis of formazan from (3-amino-2-sulfanyl-2,3,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]pyrimidin-4(1*H*)-one)

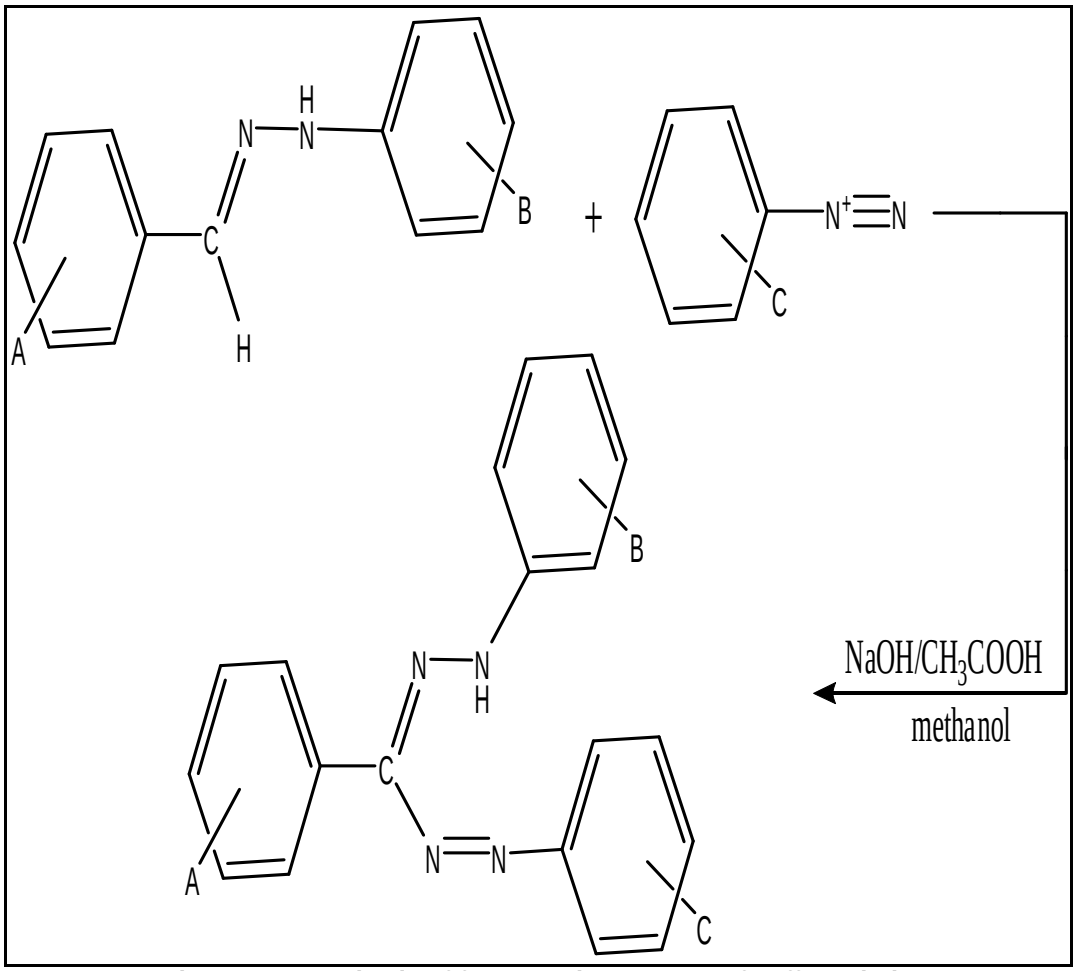


Scheme. 12: synthesis of Bis-substituted formazan

As general, coupling reactions are limited by the availability and stability of the specific arylhydrazine and aryldiazonium reagents. Therefore,the coupling reaction can occur in presence of

a)Sodium acetate and pyridine(Jasim, 2011, Hassner and Namboothiri, 2012), This method is widely used in literatures(Patel *et al.*, 2009, Revanasiddappa and Subrahmanyam, 2010, Udhayakalaa*et al.*, 2011). It allows access to both symmetrically and unsymmetrically *N,N'*-disubstituted formazans, and is the only general method for synthesis of 1,3,5-triphenyl formazan, but it is highly cumbersome and resulting products are poor and difficult to purify. In addition of the toxicity of pyridine solvent and seriously limits scale up(Katritzky *et al.*, 1995).

b) Sodium hydroxide, sodium carbonate hydrate, and methanol(Tezcan and Özbek, 2005, Abdel Ghani*et al.*, 1987), (Scheme 13). Tezcan and Özbek preferred this way (in spite of the difficulty of purification and the low yield) because of the availability of the starting materials in the laboratory, and they tried to increase the yield in order to provide a cheap way to synthesize formazan for medical, dying, and analytical applications by using buffer solution (Tezcan and Özbek, 2005). Finally, they found that the best yield of formazan was in basic buffer solution of (NaOH + CH_3COONa) with (pH 10-12).



Scheme. 13: Synthesis of formazan in presence of puffer solution

c)Under phase-transfer conditions(Katritzky *et al.*, 1995, Gilroy, 2008), By using tetrabutyl ammonium bromide or dicyclohexano-18-crown-6 (phase transfer catalyst, PTC), dichloro methane, mild base (sodium or potassium carbonate) and water, (Scheme 14).

Azo-coupling of aryldiazonium salt with phenylhydrazones under mild basic conditions in two-phase liquid-liquid media is efficiently promoted by PTC at 5-25 °C. The condensation of benzaldehyde with phenylhydrazine followed phase-transfer catalyzed azo-coupling with phenyl diazonium chloride gave 1,3,5-triphenyl formazan.

The disadvantages of diazonium salt such as instability and insolubility in low polar media can be exceeded by solid-liquid phase transfer catalyzed, and the reaction is significantly accelerated by PTC.

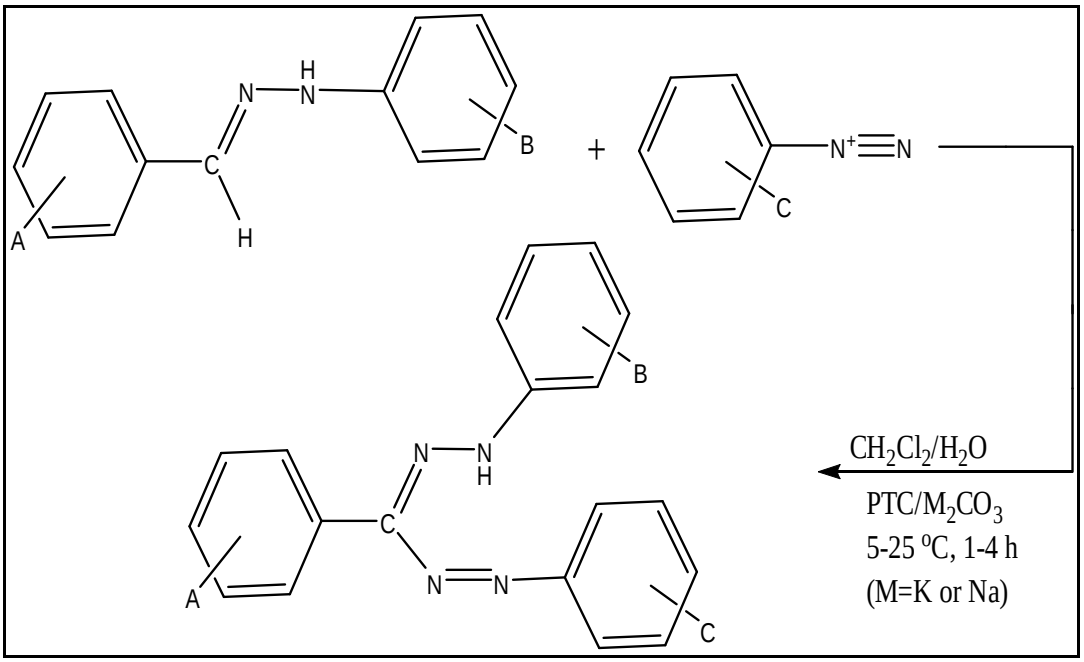
Katritzky*et al.* (Katritzky *et al.*, 1995),showed that there are three important experimental details should be noted:

a) The reaction proceeds at temperatures which are higher than normally required for azo-coupling.

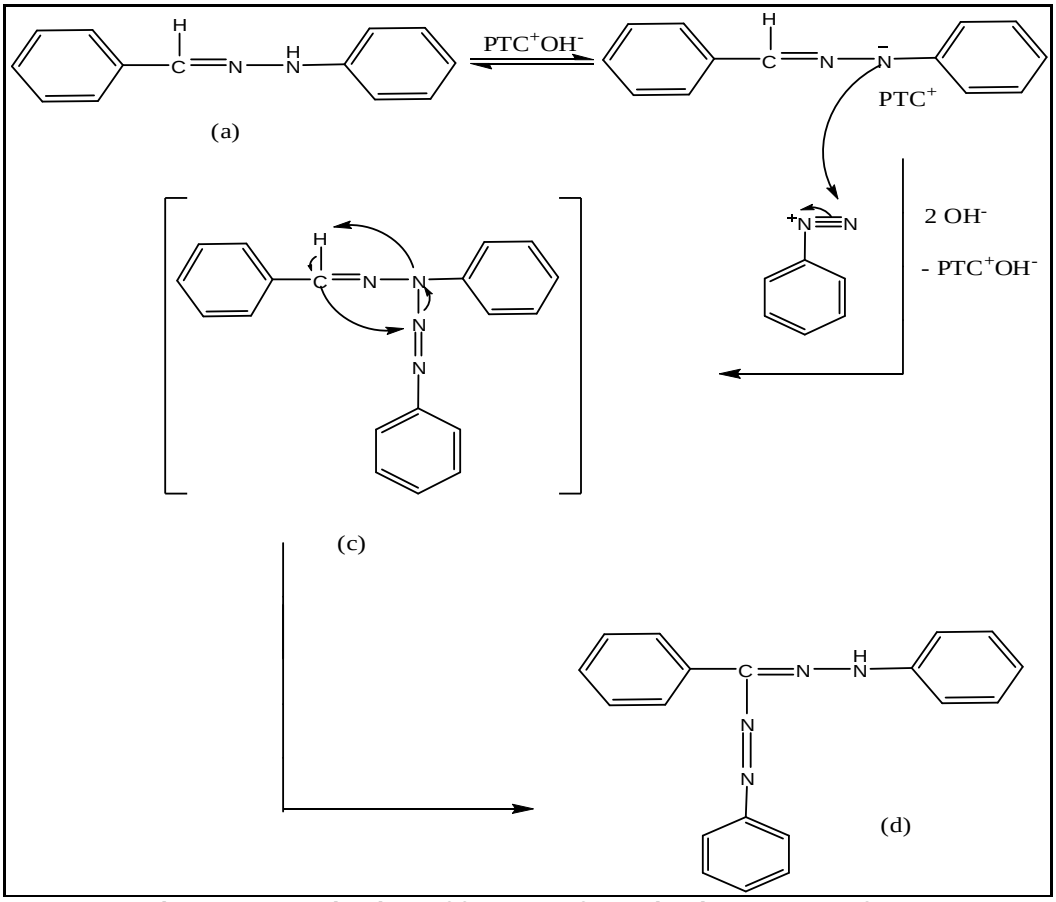
b) The reaction is not as exothermic as in the case of azo-coupling in pyridine.

c) The basicity of aqueous phase is lower than in the traditional pyridine synthesis.

Also, they described the mechanism of this reaction by showing that the possible mechanism based on the theory of PTC, which assume that interactions proceeds in water phase. The anion formation under the action of mild base (Sodium or potassium carbonate) occurring at the more acidic (NH) rather than at the (CH)of hydrazone (a). The ambident anion thus formed could either azo-couple at the nitrogen atom with subsequent rearrangement of the unstable tetrazene (c) into formazan (d) or give (d) directly, (Scheme 15).



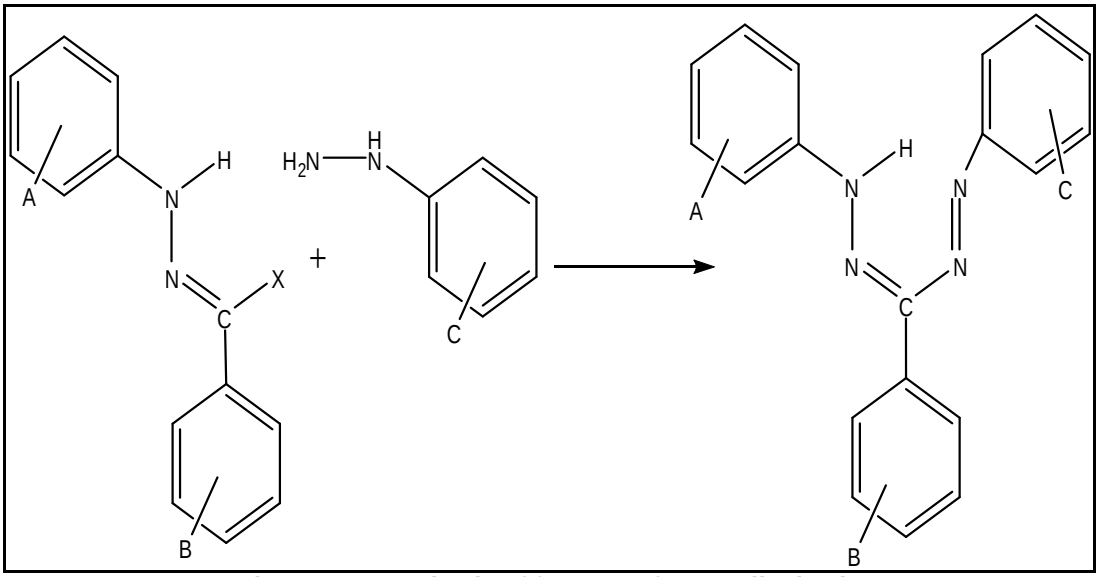
Scheme. 14: Synthesis of formazan under bi-phasic condition



Scheme. 15: Mechanism of formazan formation in presence of PTC

4.2. Reaction of Halogenated Schiff BaseWithArylhydrazine(Gilroy, 2008)

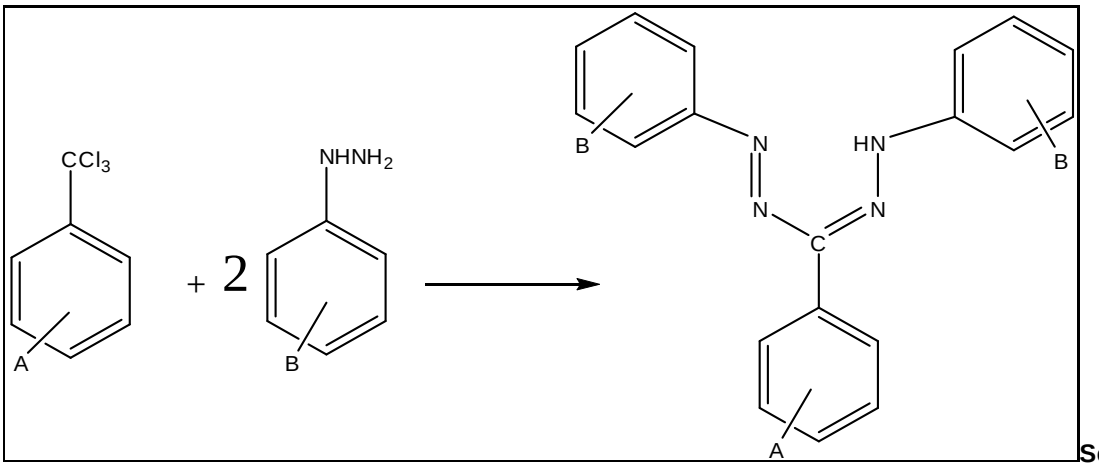
This synthesis do not rely on the use of diazonium salts (Scheme 16), and used to prepare triphenyl formazans.



Scheme. 16: Synthesis of formazan from arylhydrazine

4.3. Reaction of Tow Equivalents of Arylhydrazine and Benzotrichloride(Borsche and Manteuffel, 1933)

Also, this method does not rely on the use of diazonium salts (Scheme 17), and used to prepare triphenyl formazans.



Scheme. 17: Synthesis of formazan from tow equivalents arylhydrazine

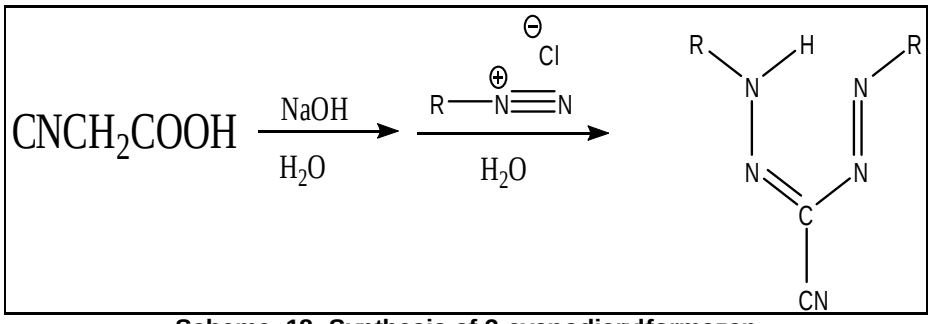
4.4. Reaction of Tow Equivalents of Diazonium Salt and Methylene Precursors

4.4.1. Synthesis of 3-Substituted Diphenyl Formazan

3-Substituted diphenyl formazans are not as common as triphenyl formazans, but can be synthesized by alternative method that do not rely on the synthesis of Schiff bases.

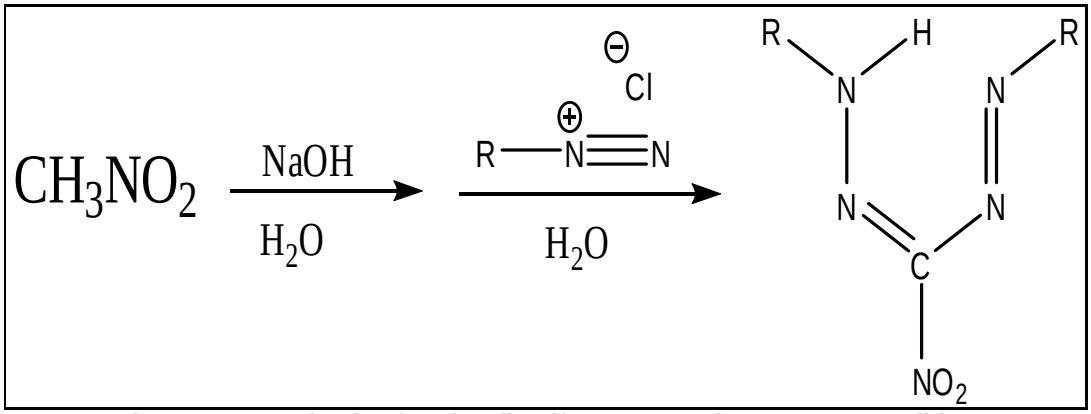
The coupling of active methylene compounds with two moles of diazonium cations is highly practical and easy but only gives symmetrical formazan, and the substituents at the carbon atom are limited to non aromatic structures. Yields depend strongly on the type of substituents at the active methylene group (cyano, nitro or carboxy). The basicity of the medium, ratio of reagent, and type of substituents play a key role in this reaction(Katritzky *et al.,* 1995).

Depending on substituents at the active methylene group, Formazan can synthesize with either cyano or nitro groups on position (3), for this reason 3-substituted diphenyl formazan can synthesized from:
a) Cyano chloromethane ($ClCH_2CN$), and cyanoacetic acid ($CNCH_2COOH$)(Katritzky *et al.,* 1995) to prepare 3-cyano diarylformazans in presence of NaOH and H_2O.
3-Cyanoformazans(Gilroy, 2008), were synthesized via reaction of two equivalents of the appropriate aryldiazonium chloride salt with deprotonated cyanoacetic acid (Scheme18). The reaction proceeded upon attack of the diazonium cation by the *in situ* generated carbanion of cyanoacetic acid. The hydrazone-type intermediates are then deprotonated (by hydroxide or initial carbanion) and the resulting carbanion attacks a second equivalent of aryl-diazonium cation. The carboxyl group associated with cyanoacetic acid must also be lost during the reaction (probably as CO2), although it is not clear at which stage this occurs.



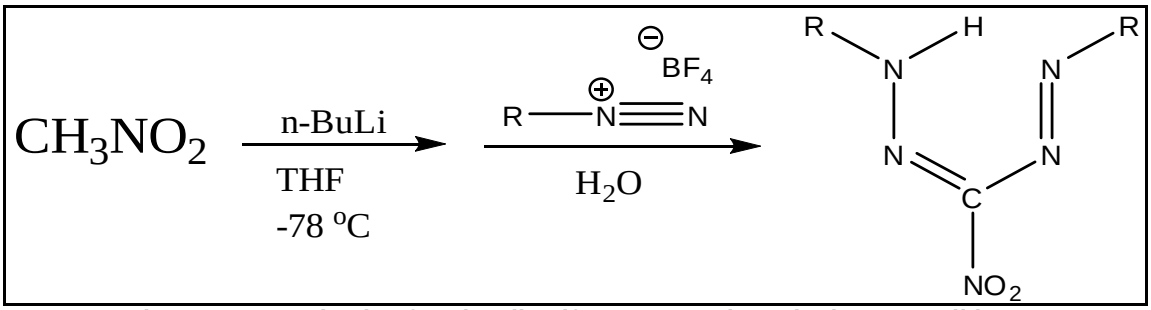
Scheme. 18: Synthesis of 3-cyanodiarylformazan

b) Nitro methane (CH_3NO_2) to prepare 3-nitro diarylformazans underaqueous or anhydrous conditions (Gilroy, 2008)
I) Aqueous conditions
3-Nitroformazans were synthesized by Gilroyby using similar methodologies to the synthesis of 3-cyanoformazans, using nitromethane instead of cyanoacetic acid (Scheme 19). This route was employed for only (R= phenyl or p-toloyl)which conveniently precipitate from aqueous solution. However, attempts to synthesize 3-nitroformazans bearing bulky N-substituents this way were not successful. In the nitromethane systems even the presence of *ortho*-methylgroups causes the reaction to fail.



Scheme. 19: Synthesis of 3-Nitrodiarylformazan under aqueous conditions

II) Anhydrous conditions
In order to produce 3-nitroformazans with bulky aryl substituents, anhydrous synthetic routes were developed. The tetrafluoroborate salts of the aryldiazonium cations were stable enough under such conditions to couple with deprotonated nitromethane producing 3-nitroformazans in reasonable yields (Scheme20).

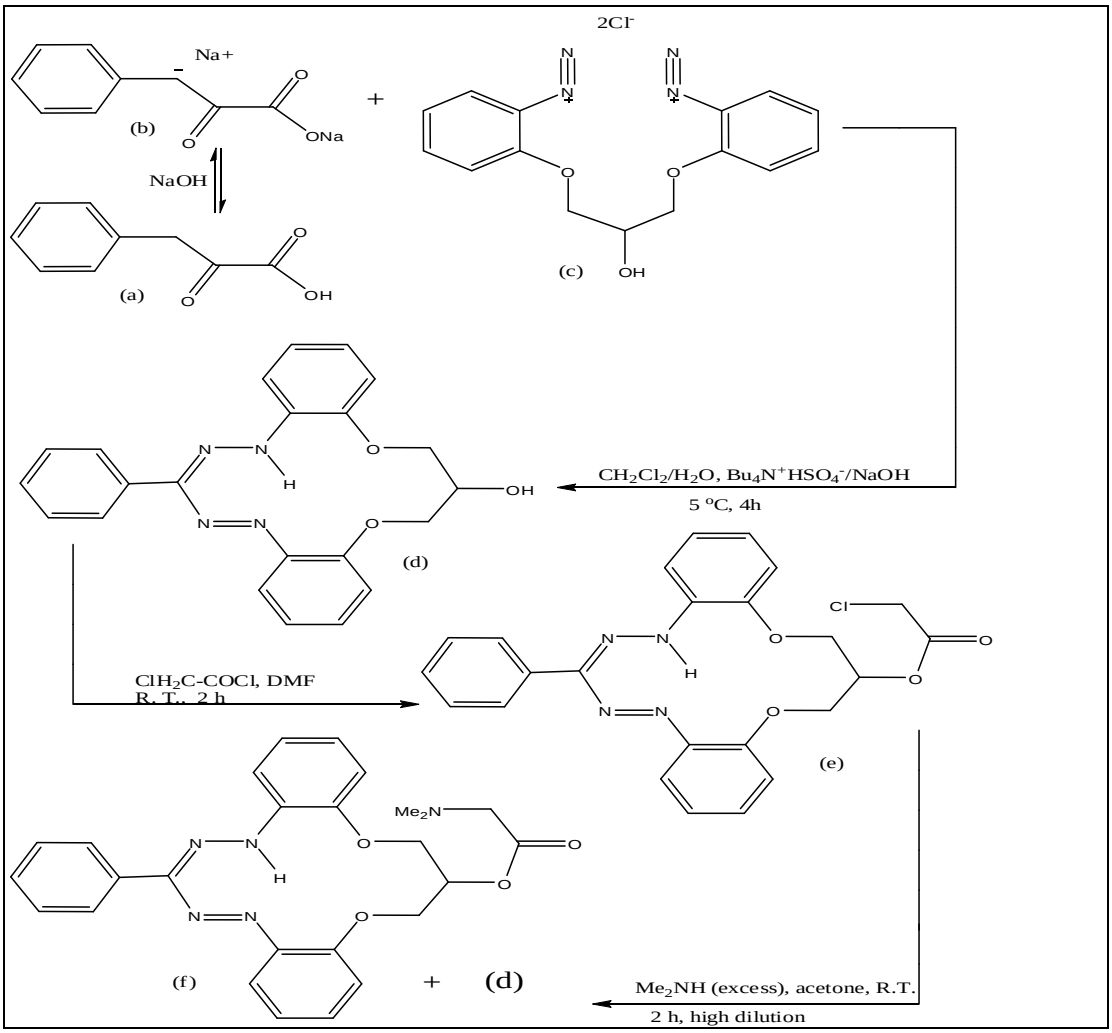


Scheme. 20: Synthesis of 3-Nitrodiarylformazan under anhydrous conditions

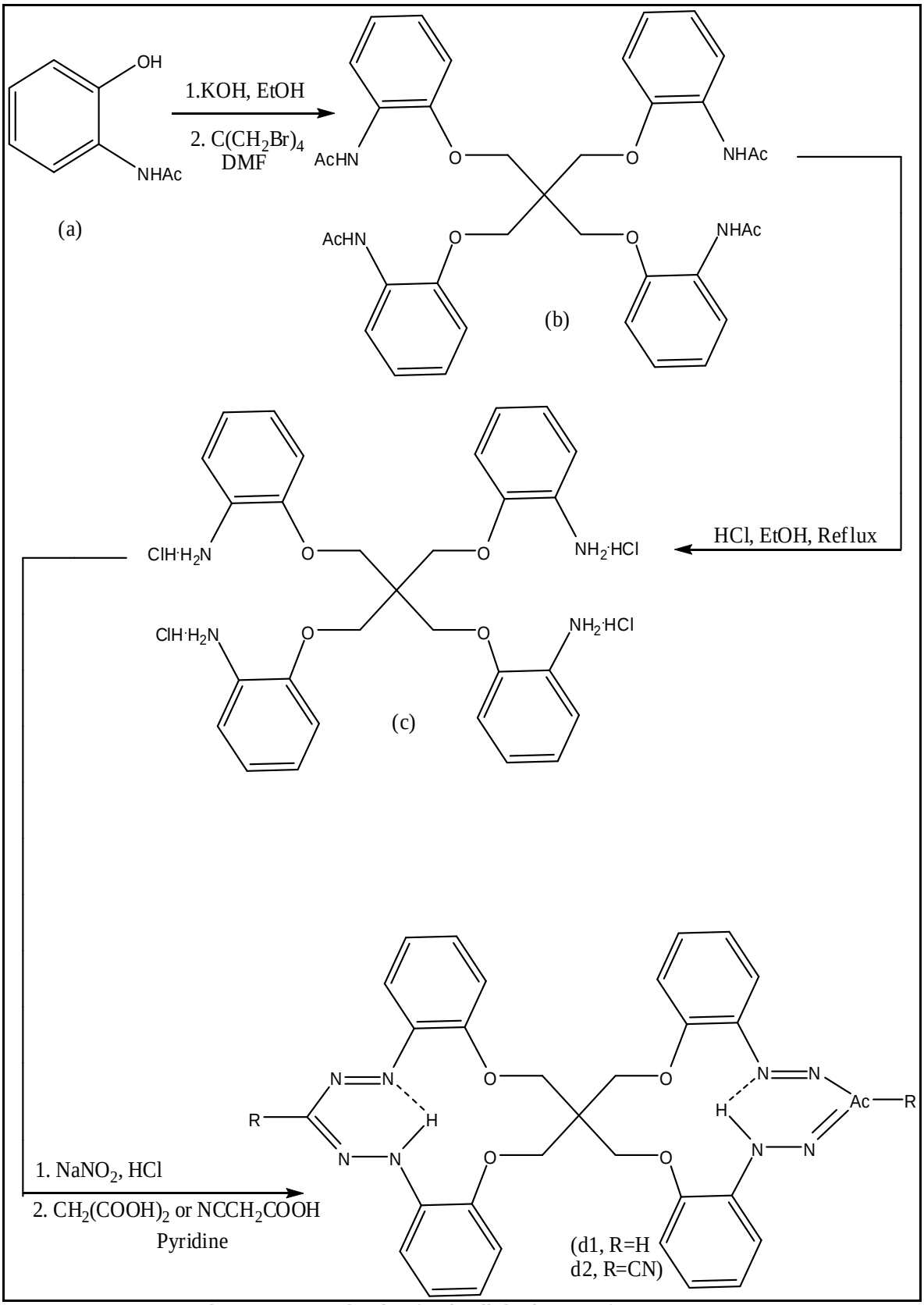
4.4.2.Synthesis of Crown Formazan

Katritzky *et al.* (Katritzky*et al.*, 1994), synthesized the first lariat crown formazan (depending on the concept of lariat ether) with a pendant hydroxy group by using a phase transfer assisted azo-coupling reaction with the active methylene (Scheme21), and this lariat crown formazan containing a strong donor group as a supporting ligand. also they found that high rigidity of the formazan in such macrocycles results in the weakening of complexing ability by reduction of the cavity size due to intramolecular hydrogen bonding and the shortening of bond distance because of strong conjugation.

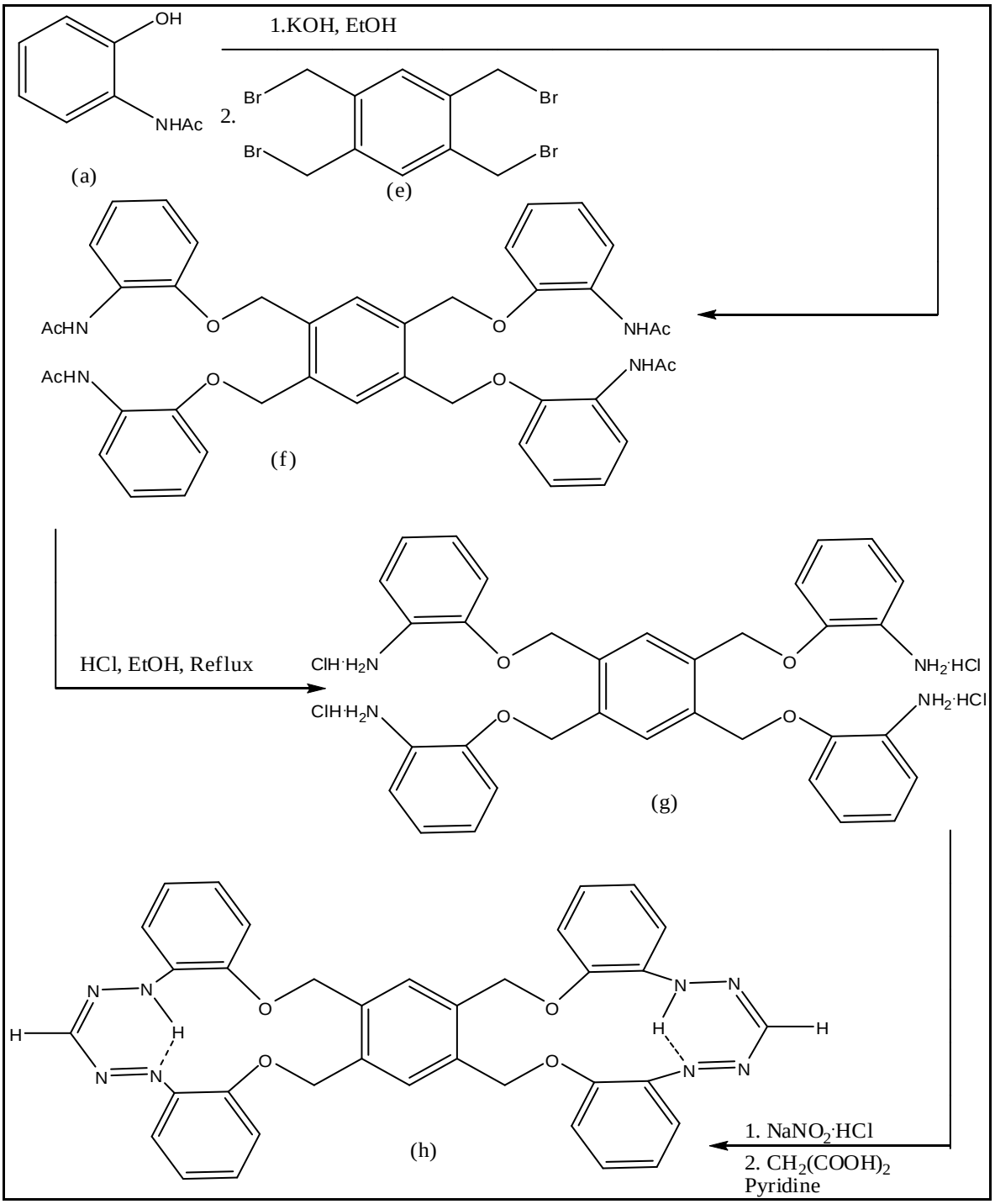
Abbas and Elwahy (Abbas and Elwahy, 2009), synthesized the spiro-linked crown formazans **d1,2**and Bis(crown formazan)**h**by diazotization of Tetrakis(amine) hydrochlorides **c,** and **g**, respectively, followed by coupling with malonic acid or cyanoacetic acid in pyridine, (Scheme22 and 23).



Scheme. 21: Synthesis of lariat crown formazan



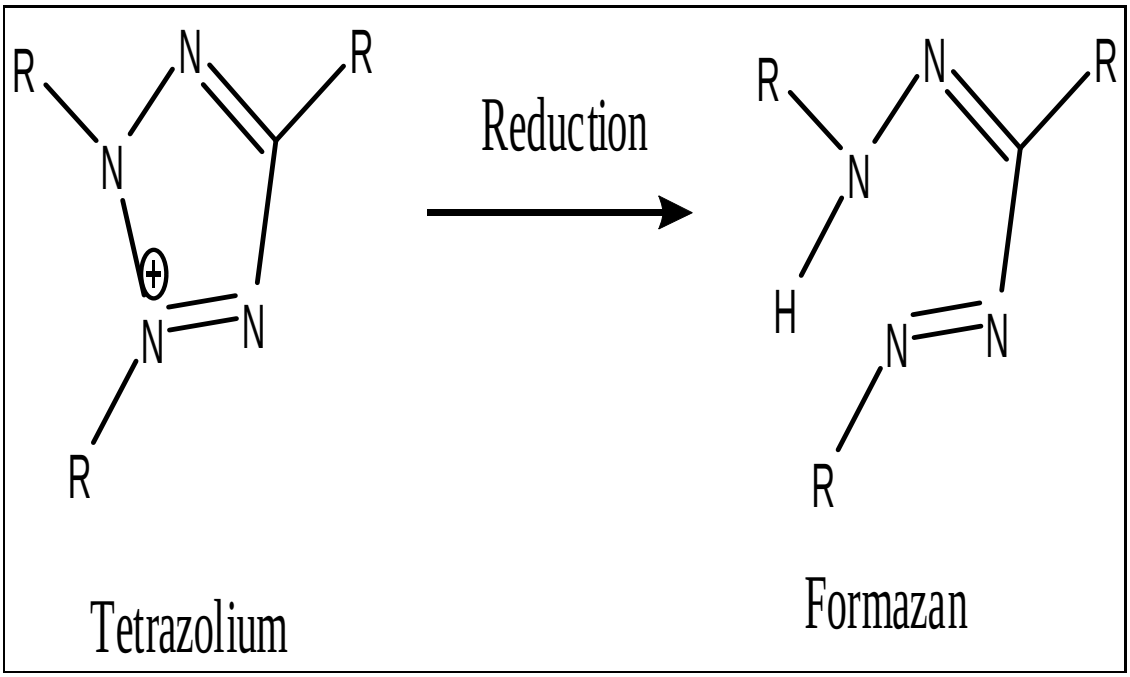
Scheme. 22: Synthesis of spiro-linked crown formazans



Scheme. 23: Synthesis of Bis(crown formazan)

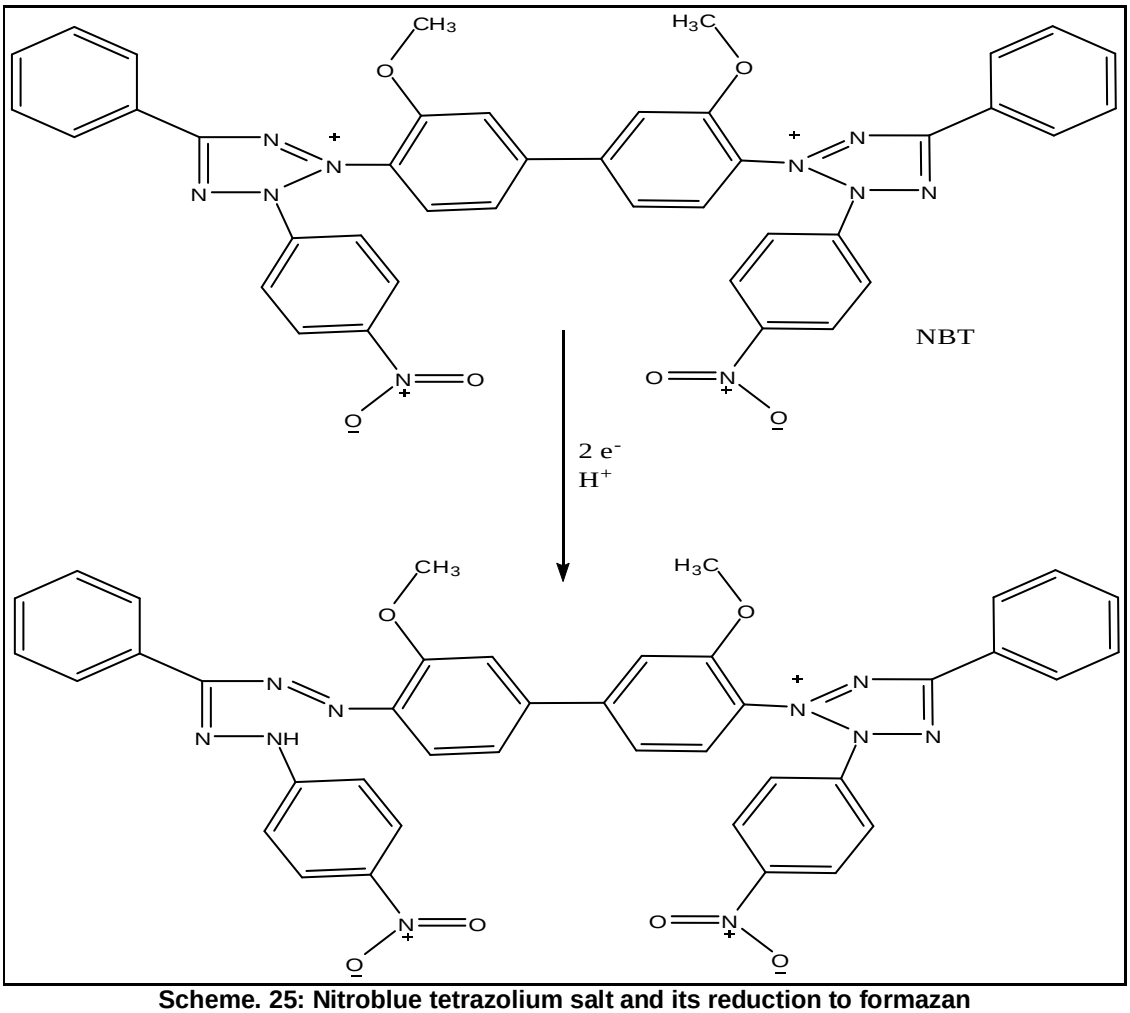
4.5. Reduction of Tetrazolium Salt

Reduction of various tetrazolium salts by dehydrogenases of metabolically active cells leads to production of highly coloured end products – formazans(Kregiel, 2012), (Scheme 24).



Scheme. 24: Reduction of tetrazolium salt

The metabolic activity of some bacterial cells is determined on the basis of quantitative evaluation of tetrazolium reductase activity of cells.Triphenylformazan (TPF) is an organic compound obtained after the reduction of triphenyl-tetrazolium chloride (TTC) and it is used mainly as an indicator for dehydrogenase activity. Due to oxidation process, the colour of formazan compound is turning from white to dark red, the intensity of its colour being proportional to the enzyme activity(Filip*et al.*, 2008).
Achilli and Grandi studied the reduction of Nitroblue tetrazolium salt (NBT) to formazan by folic acid, *N*-(4-aminobenzoyl) glutamic acid and other amino acids,and they found that the reduction involved only one of the tow tetrazolium rings of NBT. The reaction is considerably more rapid with folic acid and *N*-(4-aminobenzoyl) glutamic acid than with other amino acids(Achilli*et al.*, 2014), (Scheme 25).
Tetrazolium salts are colourless compounds which become coloured when reduced to formazans. Traditionally used as indicators of cell metabolism in eukaryotes and prokaryotes, they function as artificial electron acceptors and thus detect dehydrogenase activities. The production of coloured formazan is irreversible and can be quantified using spectrophotometry.
As shown in (Figure 2), Tachon*et al.* (Tachon*et al.*, 2009), identified the reduction mechanisms of tetrazolium violet (TV) in Lactococcuslactis using a mutagenesis approach, under two experimental conditions generally applied in microbiology: a plate test with growing cells, and a liquid test with non-growing (resting) cells. The results showed that in both tests, TV reduction resulted from electron transfer from an intracellular donor (mainly NADH) to TV via the electron transport chain (ETC), but the reduction sites in the ETC depended on experimental conditions. Using the plate test, menaquinones were essential for TV reduction and membrane NADH dehydrogenases (NoxA and/or NoxB) were partly involved in electron transfer to menaquinones. In this case, TV reduction mainly occurred outside the cells and in the outer part of the plasma membrane. During the liquid test, TV was directly reduced by NoxA and/or NoxB, probably in the inner part of the membrane, where NoxA and NoxB are localized. In this case, reduction was directly related to the intracellular NADH pool. Based on these findings, new applications for TV tests were proposed, such as NADH pool determination with the liquid test and the screening of mutants affected in menaquinone biosynthesis with the plate test. Preliminary results using other tetrazolium salts in the plate test showed that the reduction sites depended on the salt.



Scheme. 25: Nitroblue tetrazolium salt and its reduction to formazan

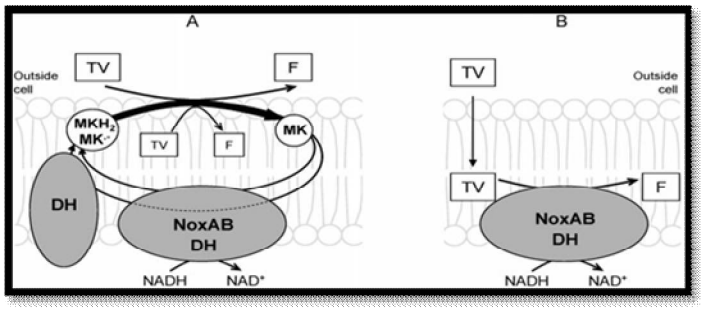


Fig. 2: Schematic representation of the mechanism of tetrazolium violet (TV) reduction: (A) in the plate test with growing cells; (B) in the liquid test with resting cells. TV:tetrazolium violet; F:formazan; MK: oxidized menaquinones; MK:semiquinones; MKH2: reduced menaquinones; NoxAB DH: membrane NADH dehydrogenases NoxA and NoxB; DH: dehydrogenase(Tachon*et al.*, 2009).

Kaprelyants and Kell described 5-cyano-2,3-ditolyl tetrazolium chloride (redox dye) which reduced to a fluorescent formazan derivative. Also, described its use together with flow cytometry for the visualisation of respiratory activity in individual cells of *Micrococcus luteus*(Kaprelyants and Kell, 1993).

A photocurrent response mechanism of quantum dots (QDs) under illumination with the concept of quantum photoelectric effect is presented by Wang *et al.* (Wang *et al.*, 2013). Upon irradiation, the photoelectron could directly escape from QDs. By using (NBT) to capture the photoelectron, a new

visual system was proposed due to the formation of an insoluble reduction product, purple formazan, which could be used to visualize the quantum photoelectric effect, (Figure 3).

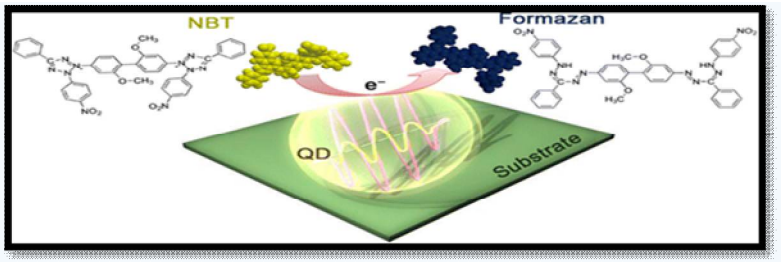


Fig. 3: Reduction of NBT to formazan in presence of QDs(Wang *et al.*, 2013)

4.6. Modification of present formazan

Formazans may be converted to other formazans by effecting changes in functional groups substituted in the molecule, such as(Şenőz, 2012):

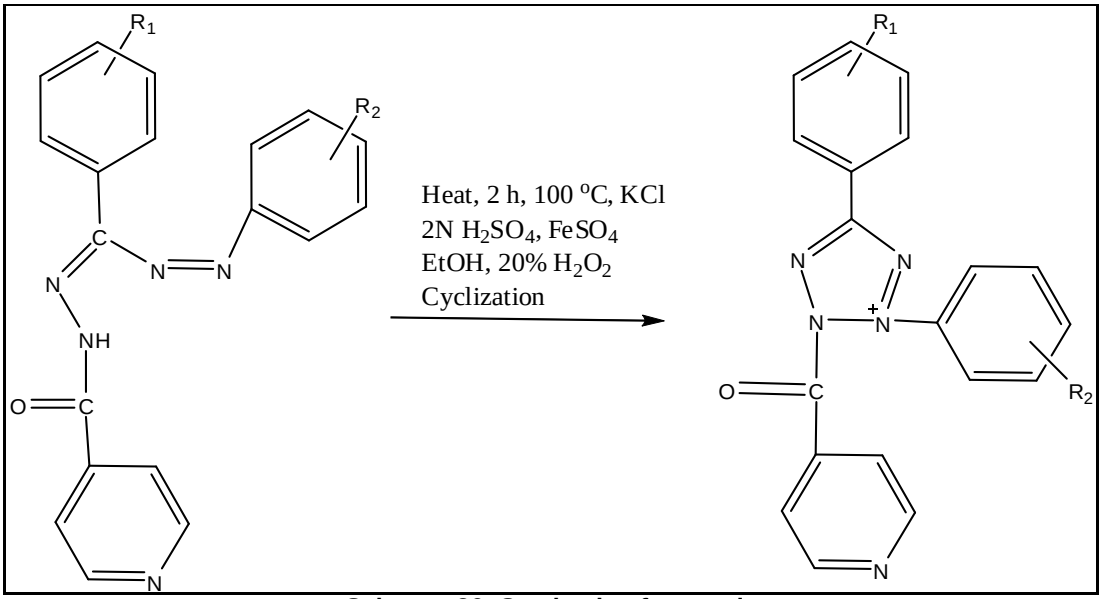
a) Hydrolysis of ester and nitrile substituents to the carboxylic acids.
b) Hydrolysis of N-acyl groups to free amino groups.
c) Reduction of nitro groups to amines.
d) Esterification of carboxylic acids through the silver salts.
e) Decarboxylation of C-carboxyl compounds.

5. Reactions of Formazan

5.1.Oxidation of Formazan in Presence of Base to PrepareTetrazole and TetrazoliumSalt(Deepa*et al.*, 2014)

Tetrazole are class of synthetic organic heterocyclic compounds consisting of five membered ring of four nitrogen and one carbon atom. The development of tetrazole chemistry has been largely associated with wide scale of applications in these compounds: medicine, biochemistry, agriculture, photography, as well as corrosion inhibitors, components of gas generating compositions, and explosives.

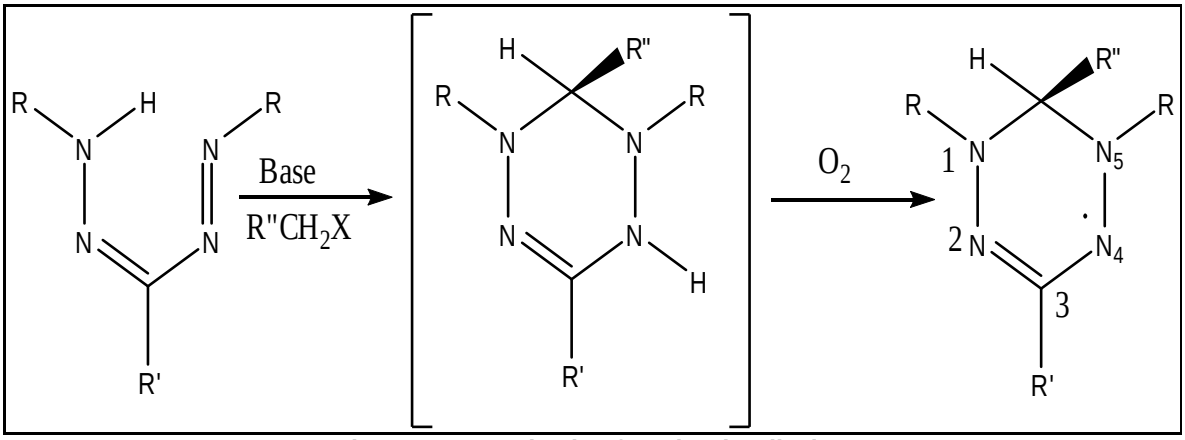
A considerable advance in the tetrazole chemistry has been made by Russian scientists. Tetrazole was first prepared by the reaction of anhydrous hydrazoic acid and hydrogen cyanide under pressure. Keeping these in mind efforts were taken to synthesize a series of new synthons bearing tetrazole nucleus by Valentina, Ilango and Deepa,(Scheme 26).



Scheme. 26: Synthesis of tetrazole

5.2.Reaction WithFormaldehyde to Prepare Verdazyl Radicaland VerdazyliumSalt

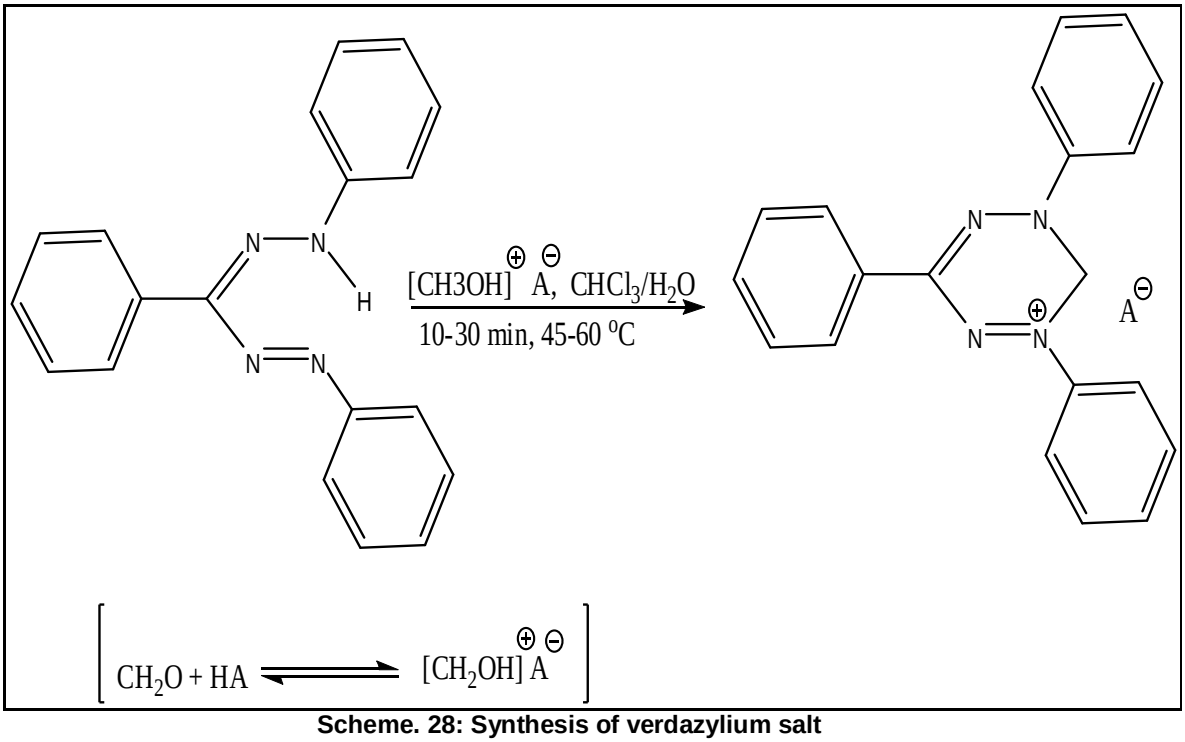
The first verdazyl radical was reported by Kuhn and Trischmann(Kuhn and Trischmann, 1963). Their serendipitous discovery occurred during the attempted alkylation of 1,3,5- triphenyl formazans. The *leuco*-verdazyls generated were spontaneously airoxidized affording corresponding 1,3,5-triarylverdazyl (type I) radicals (Scheme 27). Since then, a number of modifications to this synthesis have beenreported, generally varying the base as well as the source of bridging carbon(Katritzky and Belyakov, 1994).



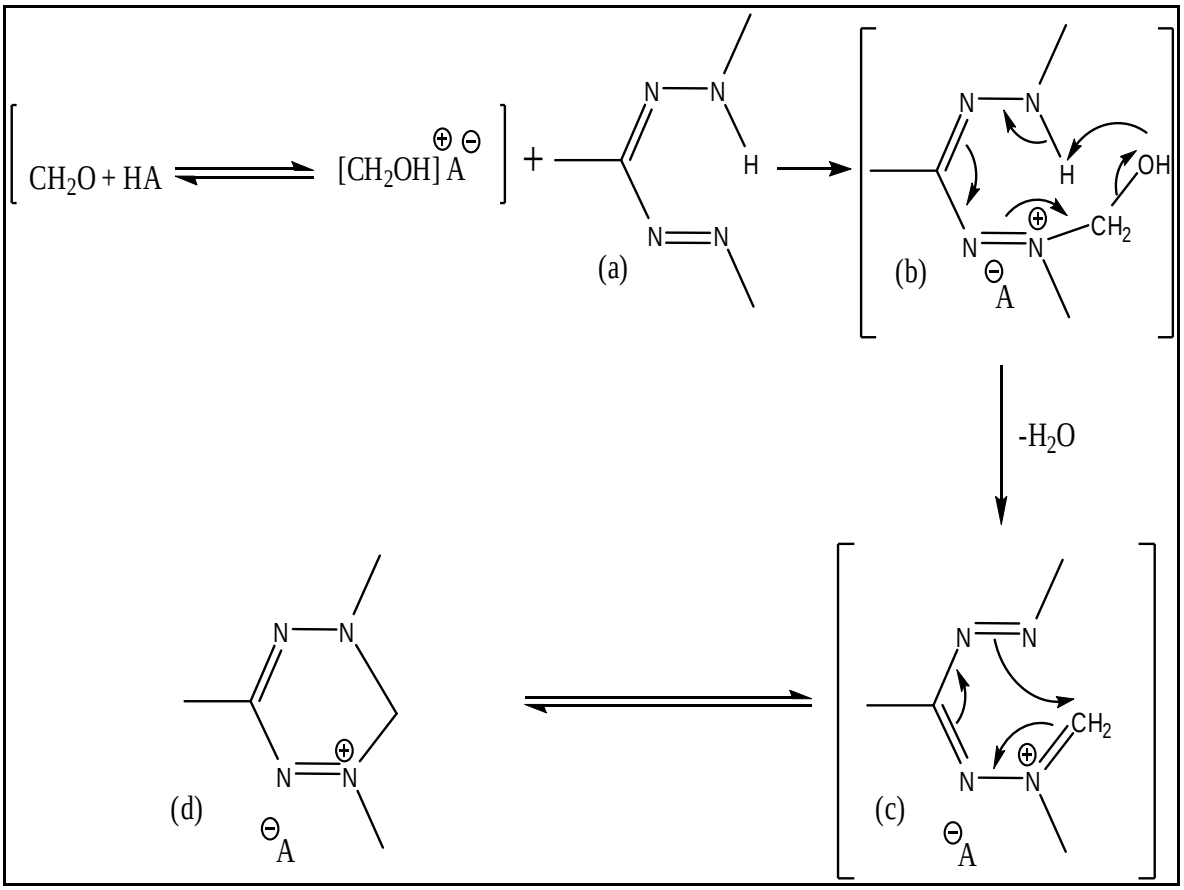
Scheme. 27: Synthesis of verdazyl radical

Published preparations of verdazyl radicals sometimes include verdazylium salt formation (Scheme 28) as an intermediate stage, when the reaction of formazan with aliphatic aldehyde is carried out in the presence of acidic catalysts. That verdazylium salt produces from the oxidation of verdazyl radical(Katritzky and Belyakov, 1994).

Katritzky and Belyakov (Katritzky and Belyakov, 1997), suggested a mechanism for the formation of verdazylium salt that protnated formaldehyde may react with the formazan molecule (a) at the water/chloroform interface to form the N-(hydroxymethyl) tetraazapentadienylium (cation b), which after dehydration gives cation (c) a ring- opened analog of vedrazolium salt (d), (Scheme 29).



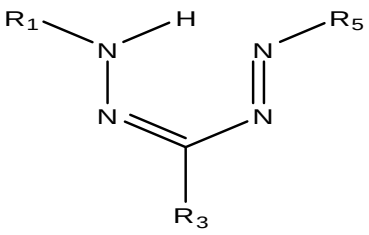
Scheme. 28: Synthesis of verdazylium salt



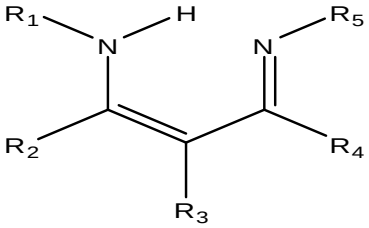
Scheme. 29: Mechanism of verdazylium salt formation

5.3.Synthesis of Metal Complexes

Gilroy *et al.* (Gilroy *et al.*, 2006), showed that formazans with close structural (I) analogues of β-diketiminates (II), possessing twoadditional nitrogen atoms in their backbone, so scientists chose them as ancillary ligand.

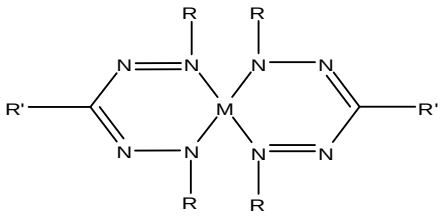


I

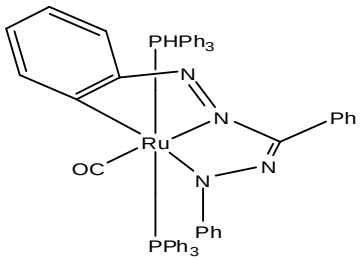


II

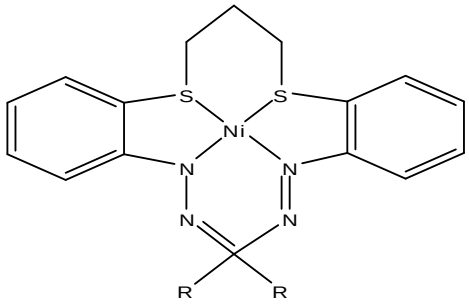
The coordination chemistry of formazans has been sporadically explored over the past 60 years, but remains an undeveloped field. Examples of systematic studies of formazan complexes are rare, and include bis formazan complexes of nickel (III)(Irving *et al.*, 1960, Dale, 1967), cyclometallated ruthenium complexes (IV)(Doff, 2012) and nickel complexes of sulfur containing macrocyclicformazans(V)(Alcock and Tasker, 1972).



III



IV



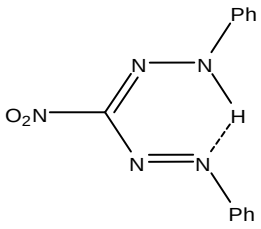
V

Transition metal complexes of polyazo ligands have attracted considerable attention in the last twenty years. Despite the fact that synthetic route to formazans were well developed over a century ago, and there are now over thousandsof reported derivatives, the coordination chemistry of formazans has not received much attention. Publications describing transition metal-formazans complexes have appeared sporadically since the 1940. There have been almost no systematic investigation. Also there is little known about the structure of metal complexes of 3-nitroformazans, although the metal-binding properties of these ligands are associated with colour changes, and as a result found applications as dyes or as metal-sensing agents(Netikma*et al*., 2014).

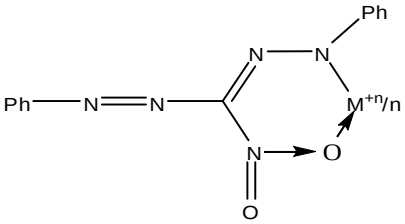
Gilroy(Gilroy, 2008), prepared complexes of (Fe^{3+}, Co^{3+}, Ni^{2+}, and Pd^{2+}) metals and used 3-Substituted diphenyl formazans (3-cyano and 3-nitro formazan) as ligands.

Nitika*et al*. (Netikma*et al*., 2014), prepared 3-Nitroformazans by reacting diazotized aromatic amine with nitromethane in cold are known to be intramolecularly hydrogen bonded. And they investigated that NH group is strongly chelated and a symmetrical structure (VI), and the experimental section for the synthesis of cobalt(III) chelates of 3-nitro-1,5-diarylformazans is being reported for the first time by them. also they found that the symmetrical 3-nitro-1,5 -diarylformazans are the versatile complex forming agents as their coordination to cobalt(III) may occur in number of ways, because of the large number of coordination centers in them. The chelation of (VI) with cobalt(VIII) may occur through:

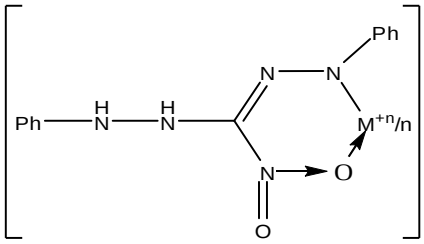
1) One of the oxygen atoms of the nitro group and one of the four nitrogens of the formazans group giving structures (VII) and (VIII).
2) Nitrogens 1 and 4 of the formazans group giving structures (IXa) or (IXb) having cis or trans geometry respectively.
3) Nitrogen 1 and 5 of the formazan group giving symmetrical six membered chelates ring structure (X).
4) Balt*et al.* (Balt*et al.*, 1977), reported kinetic study of complex formation between copper(II) and l-(2-hydroxyphenyl)-3,5-diphenylformazan in an ammoniacal ethanol-water mixture has brought evidence for a stepwise coordination of the tridentate ligand to the metal ion. Also, they (Balt*et al.*, 1980) reported kinetics and mechanism of complex formation between 1-(2-hydroxyphenyl)-3,5-diphenylformazan and ammineaquo nickel(I1) complexes, and they found that intermediate (XI) is isolable which in contrast to the more stable isomeric form (XII) has five membered formazan ring.



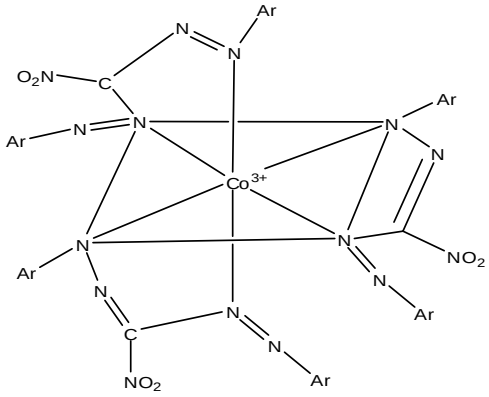
VI



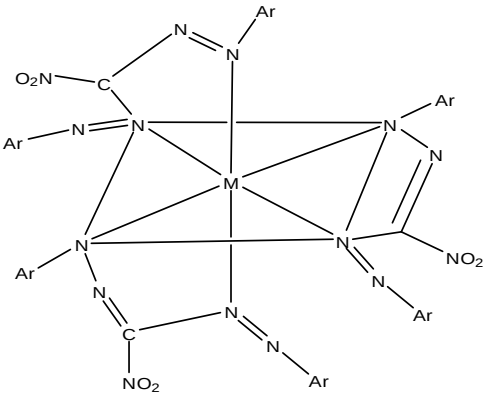
VII



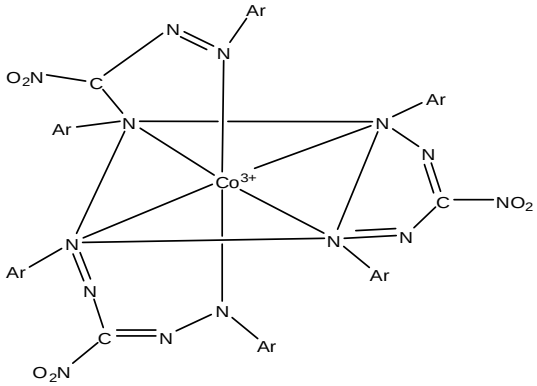
VIII



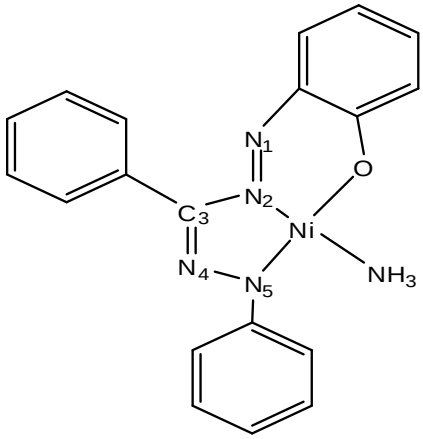
IXa



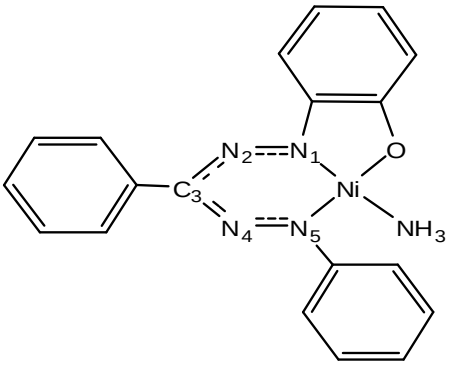
IXb



X

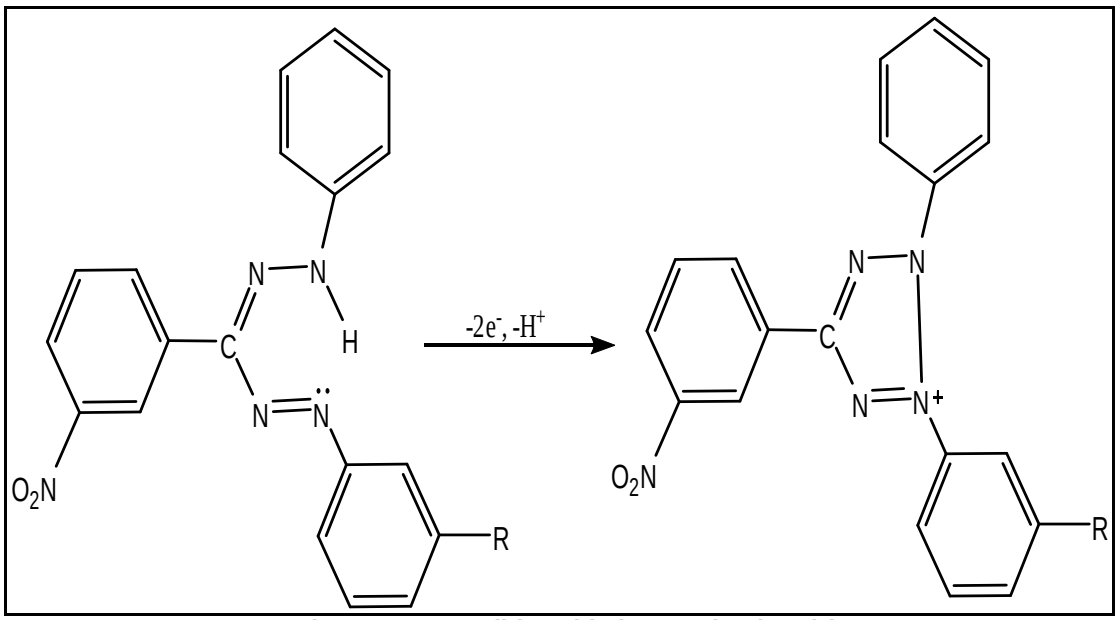


XI

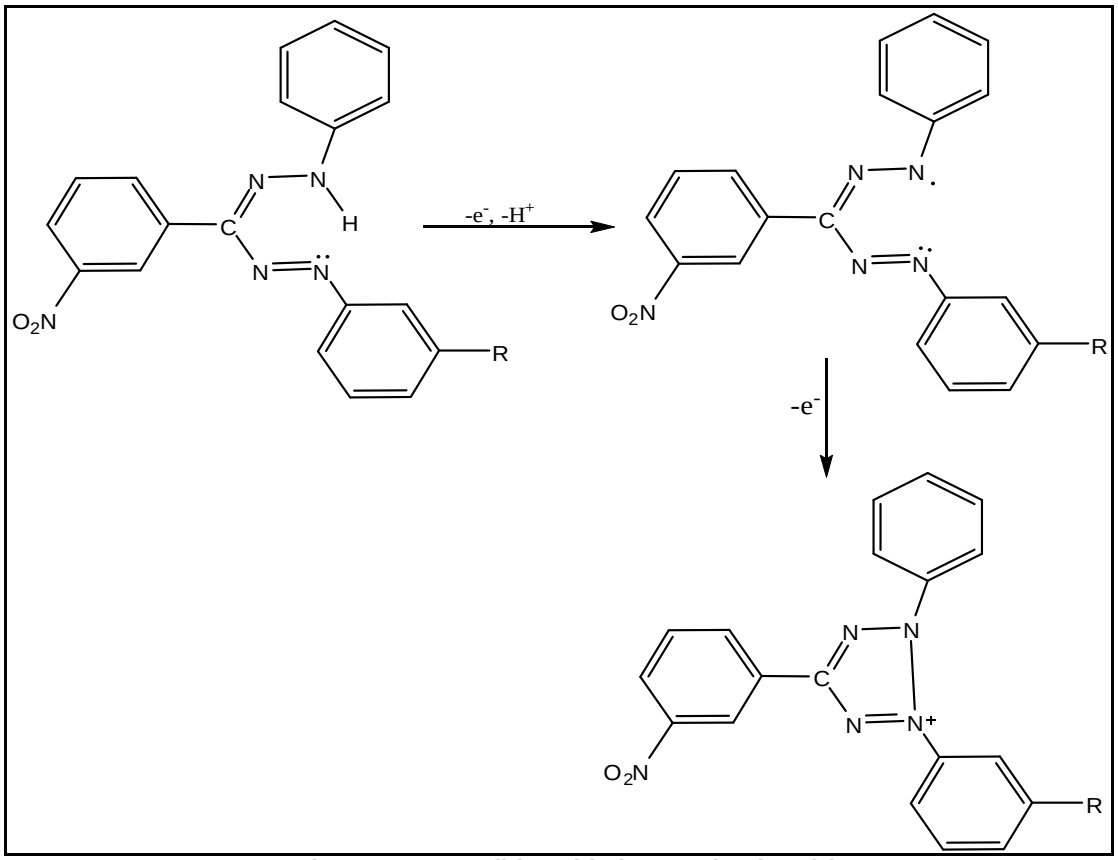


XII

Uchiumi*et al.*(Uchiumi*et al.*, 1991), studied metal complex formation of some anthrylformazans, and their investigation involved the following metal ions: copper(II), zinc(II) and cadmium(II).
Nickel(II) complexes of formazans were prepared and characterized by Tezcan*et al.* (Tezcan *et al.*, 2008, Tezcan *et al.*, 2010). Their absorption and redox behavior were discussed. Their peak potentials (E_{ox}, E_{red}), diffusion coefficients and a number of electrons transferred (n) were determined. Mechanisms of oxidation of compounds showed the effect of changing the type and the position of the substituent on the rings.Spectroscopic characteristics of the prepared complexes are discussed. Heterogeneous rate constants (k_s) were obtained. The oxidation mechanism was occurred in a single-step two electron (1),(Scheme30),or two steps two electron (2) transfer to forming tetrazolium salts, (Scheme 31). Additional support to the above proposed electro-oxidationscheme was found to be of importance.



Scheme. 30: Possible oxidation mechanism (1)



Scheme. 31: Possible oxidation mechanism (2)

Tezcan and Özbek(Tezcan and Özbek, 2005), investigated that the formation of Fe complexes was dependent upon the pH value and it was observed that there were two different isomers formed at pH 2 and pH 7–8.

6. Applications of Formazan

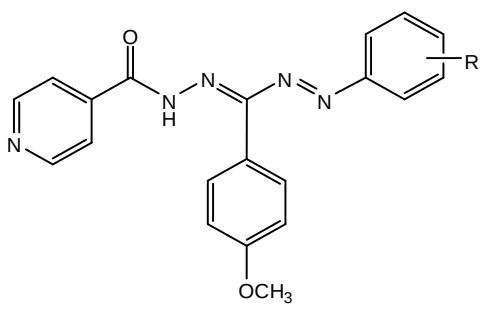
6.1. Biological Activities

During the last few years the potential of Schiff base derivatives in pharmaceutical and medicinal field have been subjected to investigation. Also, Schiff bases are utilized as starting material in the synthesis of pharmaceutically important compounds such as formazan derivatives.

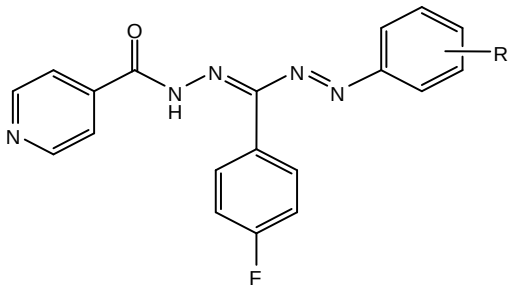
In recent decades, the problem of microbial infections has reached alarming levels in the developing countries round the world. Studies on the influence of structure on activity showed that sometimes, minor changes in the nuclei enhance the pharmacological profile multifold than the parent molecule. The search for new, effective and safe nuclei has led to improvements in the existing drugs by increasing their potency, duration of action as well as minimizing their toxic effects. This is achieved by creating new biologically active agents by molecular modifications. Azomethines and their derivatives have been prominent research subject due to their striking complexometric behaviour and pharmacological characteristics. These properties allow them to play pivotal roles in various biological activities[25].

Raval *et al.*Synthesized, Characterized and determined *in vitro* antibacterial activity of 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans (XIII)(Raval *et al.*, 2009), and 3-(4-flourophenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans (XIV)(Raval *et al.*, 2009). these compounds showed a good degree of inhibition for many types of gram positive organisms (*Bacillus subtilis, Micrococcus lucteus, Bacillus sphaericus,* and *Staphylococcus aureus*)and gram negative organisms (*Chromobacteriumviolaceum, Klebseillaaerogenes, Pseudomonas aeruginosa, Escherichia coli, Klebseillapneumonial, Salomonellaparatyphi A*).

Also, Raval *et al.*(Raval *et al.*, 2009), used 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans (XIII) as antibacterial against *M. tuberculosis*.

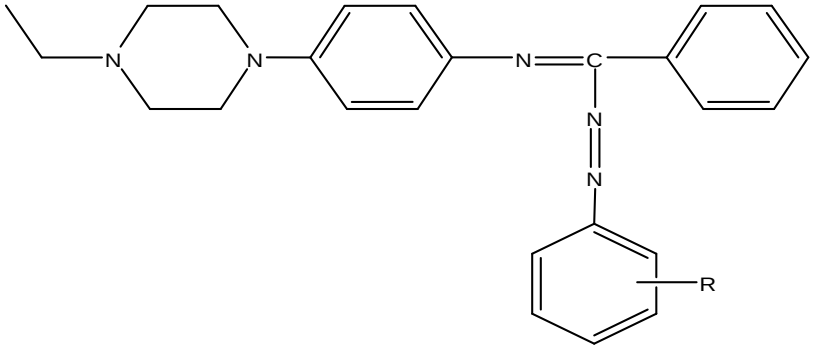


XIII



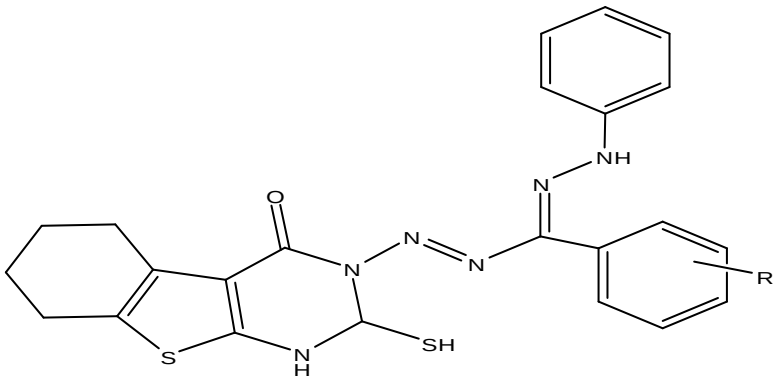
XIV

Many of scientists synthesized formazans from compounds that already have biological activity to increase the activity of formazan. Such as, Patel*et al.* (Patel*et al.*, 2009), used formazan (XV) which is derived from piperazine derivatives as antibacterial against (*Bacillus subtilis, Salomonellaparatyphi* and *Escherichia coli*)and antifungal activity against *Candida albicans.* That Piperazine derivatives have shown to possess diverse biological properties.



XV

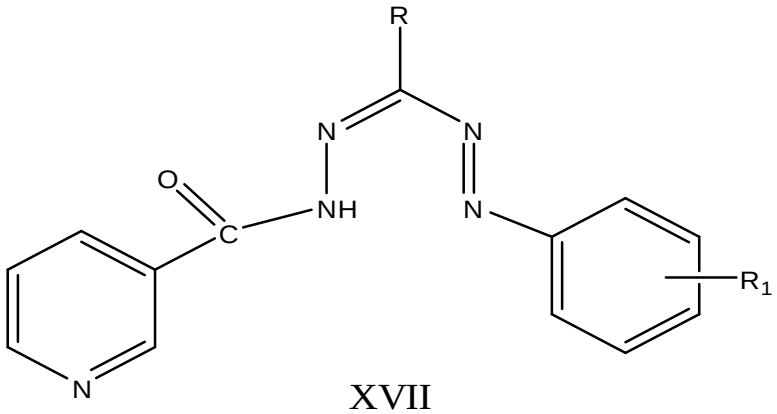
Also, Lakashmi*et al.* (Lakashmi*et al.*, 2009), used (3-amino-2-sulphanyl-2,3,4,5,6,7,8-hexahydro (1) benzothione (2,3-d) pyrimidine-4(1H)-one) (XVI) as antibacterial agent against (*Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*) and antifungal agent against *Candida albicans.* These compounds derived from thienopyrimidines which constitute the active class of the compounds possessing wide spectrum of biological activity.



XVI

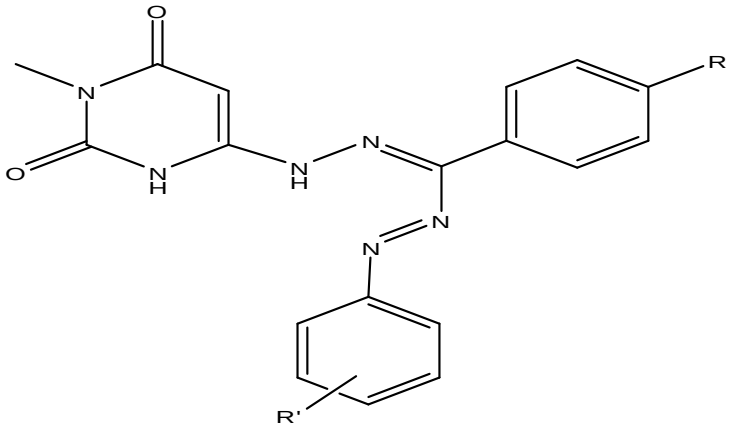
Schiff bases exhibit good antibacterial activity and pharmacological activity. These agents are one of the important synthons for the synthesis of a variety of heterocyclic compounds. Furthermore, formazans nucleus is known to be pharmacophoric in nature. According to this concept, Revanasiddappa and Subrahmanyam(Revanasiddappa and Subrahmanyam, 2010), synthesized formazans (XVII) that have antibacterial activity against (*Staphylococcus aureus, Pseudomonas*

aeruginosa, *Bacillus subtilis,* and *Escherichia coli*) and antifungal activity against *Candida albicans* and *A.niger.*



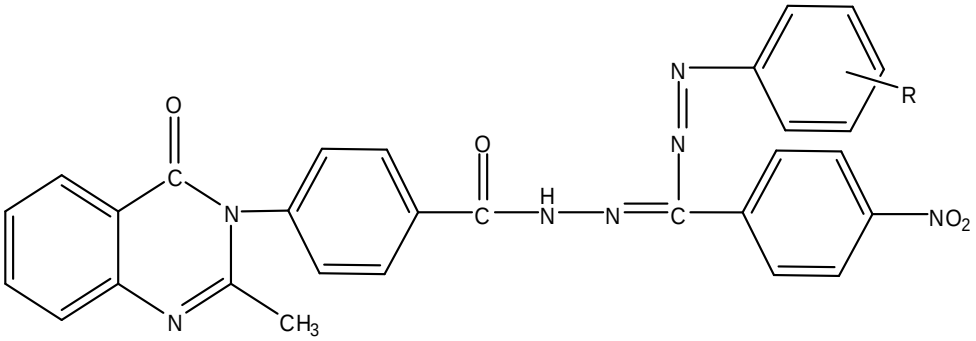
XVII

The antiviral properties of some formazan derivatives have been reported in which the antiviral effect is attributed to the presence of an intact C=N-NH grouping and C–N=N grouping, According to this concept, Mariappan*et al.*(Mariappan*et al.*, 2010), prepared and used formazan derivative as antiviral compound against *Japanese encephalitis*, but all prepared compounds did not inhibit the activity of this virus. Also, they used these compounds as antibacterial agent against (*Staphylococcus aureus*, *Escherichia coli*, *Shigellasonnei*, *Shigellaparatyphi*, and *Vibrio cholera*) and anticonvulsant agent.
The importance of uracil and its annulated substrates is well recognized by synthetic as well as biological chemist. And 6-(Arylazo) pyrimidine antimicrobials selectively inhibit replicative DNA synthesis in gram-positive bacteria by inhibiting, specifically, the replication-specific enzyme, DNA polymerase III. For this reason, Samel and Pai(Samel and Pai, 2010), prepared and used uracil formazans (XVIII) as antibacterial compound against *Staphylococcus aureus* and *Escherichia coli*, and antifungal against *S.Cerevisiaec*and *Candida albicans.*



XVIII

In addition to antibacterial activity against: (i) gram negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa)*, and (ii) gram positive organisms (*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,Staphylococcus epidermidi*), and antifungal activity against four fungal organisms (*Aspergillusniger, Saccharomycescerevisiae, Candida albicans, Candida glabrata*), Nadendla and Babu(Nadendla and Babu, 2011), determined analgesic activity for synthesized formazans (XIX).

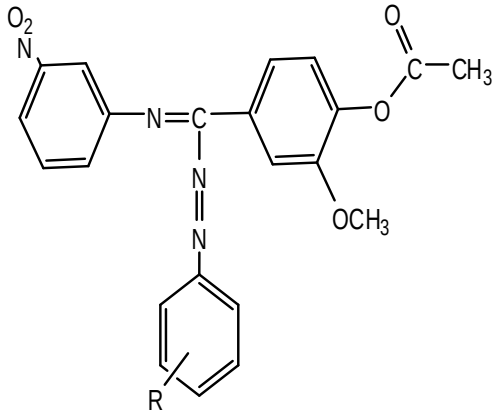


XIX

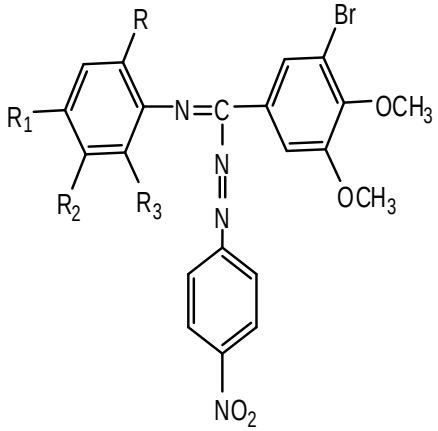
Edwards*et.al.* used azo compound and formazans dyes for the determimation of mutagenicity(Edwards *et.al.*, 2004). Stellmach prepared formazans dyes by Ehrlich ascites tumor cells(Stellmach, 1984).Sethi*et.al.*reported some formazans and tetrazolium indoles to act as CNS active agents(Sethi*et.al.*, 1984).

Halve *et al.* (Halve *et al.*, 2011), prepared 2-methoxy-4-{[(3-nitrophenyl)imino] [phenyldiazenyl]methyl}phenyl acetate (XX) and used them as antibacterial activity against (*Staphylococcus aureus, Escherichia coli, B. anthresis, S. flaxinerri*and *K. pneumonia*), and antifungal activity against (*C. albicanes, A. fumigatus, C. neoformans, A. niger*and *P. Ittalecum*). Also they concluded that:

1) Activity of the title compounds enhances on substitution.
2) Title compounds show higher activity with chloro substitution at para and ortho positions respectively.
3) Para substitution shows higher activity instead of ortho substitution. Chavan*et al.* (Chavan*et al.*, 2012), prepared formazan compounds (XXI) to determine their antibacterial activity against (*B.subtillus*and *E.coli*) and antifungal activity against (*A.niger*and *A.flavus*).

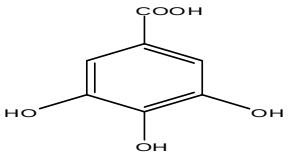


XX

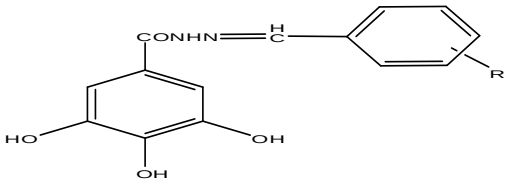


XXI

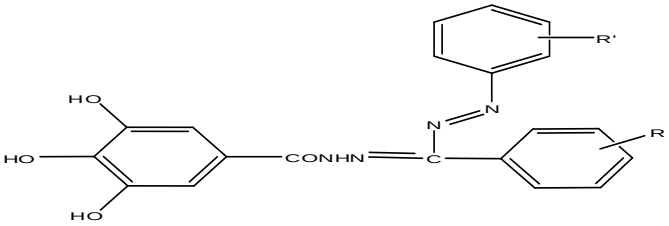
Gallic acid (3,4,5-trihydroxybenzoic acid) (XXII) is a polyhydroxyphenolic compound and found in various natural products, like gallnuts, sumac, tea leaves, oak bark, green tea, apple-peels, grapes, strawberries, pineapples, bananas, lemons, and in red and white wine1 and posses various biological activities. So Kumara Prasad*et al.*(Kumara Prasad*et al.*, 2014), used Schiff bases of Gallic acid (XXIII) to prepare formazan (XXIV) with higher bio-reactivity against bacterial organisms (*Staphylococcus aureus*and *Escherichia coli*) and fungal organisms (*Aspergillus Niger*). Also, they determined anti-inflammatory, analgesic, and anticonvulsant activities of the prepared formazans.



XXII

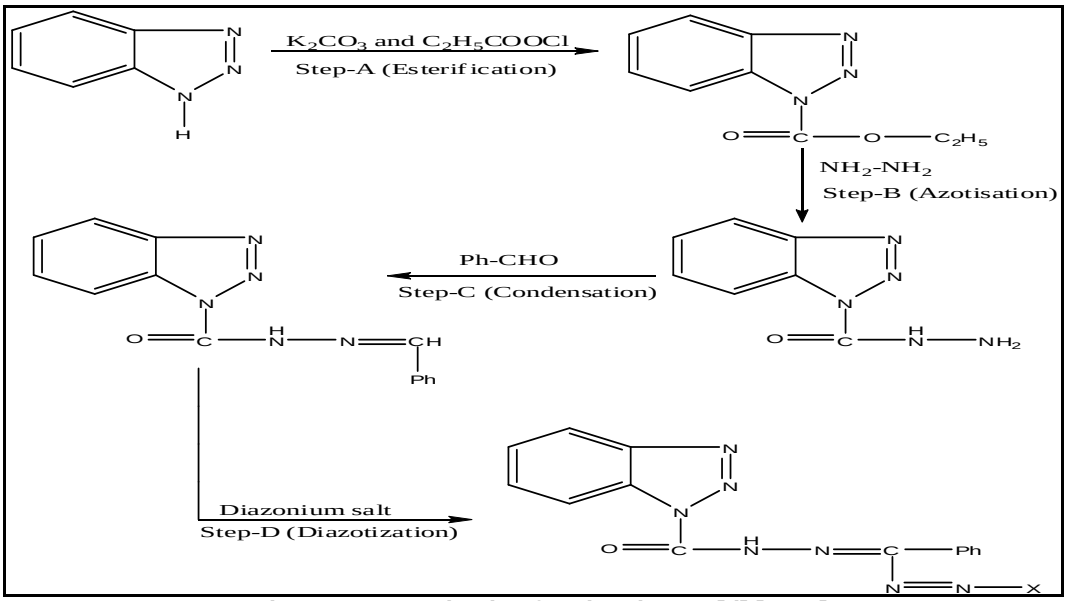


XXIII



XXIV

Also, formazan derivatives work as(anti-inflammatory, anticancer, anti-HIV, and several formazans show promising antifertility and anti-parkinsonian activity) (Shawali and Sami, 2014).
Heterocyclic compounds containing nitrogen atoms are considered to be one of the most effective antimicrobial drugs used as either single agents or in combination for cancer therapy. And some benzotriazole derivatives have shown anti-inflammatory properties. Depending on these concepts, Muvvala and Ratnakaram synthesized 1– (1H–benzo [d] [1,2,3] triazole–1–carbonyl) formazan derivatives, (Scheme 32), and studied their antibacterial activities against different strains of bacteria i.e. Gram negative organism (*Pseudonomousaureginosa*) and Gram positive organisms (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*). Some of the synthesized compounds showed significant activity against various bacteria(Muvvala and Ratnakaram, 2014).



Scheme. 32: Synthesis of 1– (1H–benzo [d] [1,2,3] triazole–1–carbonyl) formazan derivatives

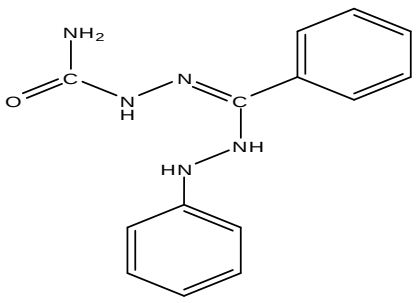
Shawali and Sami summarized the relationship between the general structural formulas of the various formazans covered in their review and the biological activities of these formazans which have been evaluated in table (1) (Shawali and Sami, 2014).

Table 1: (Shawali and Sami, 2014):The general structural formulas of the various formazans and their biological activities

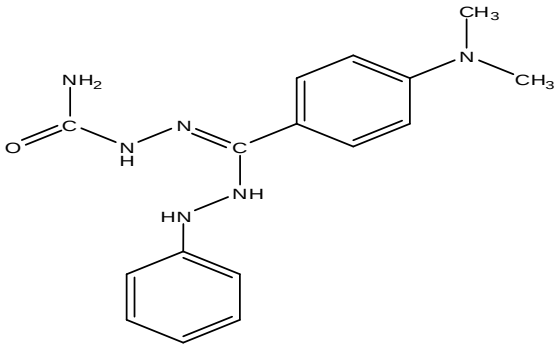
No.	General structure	Their screened biological activities
1	Ar-C(=NNHAr')-N=N-Ar	Antiparkinsonian, Analgesic, Anti-oxidant, Anticonvulsant, Antiviral, Anti-inflammatory, Antimicrobial.
2	Ar-C(=NNHAr')-N=N-Het	Anticonvulsant , Antiviral. Antimicrobial, Antiparkinsonian, Antiproliferative
3	Het-C(=NNHAr')-N=N-Ar	Antiviral
4	Ar-C(=NNH-CO-Ar')-N=N-Ar	Anthelmintic, Anti-inflammatory, Anticancer, Anti-HIV, Antimicrobial, Analgesic
5	Ar-C(=NNH-CO-Het)-N=N-Ar	Anti-oxidant, Antitubercular, Antimicrobial
6	Het-C(=NNH-CO-Ar')-N=N-Ar	Antiviral, Anti-inflammatory
7	Het-C(=NNH-CO-Ar')-N=N-Het	Antimicrobial
8	Ar-C(=NNH-R)-N=N-Ar	Anti-inflammatory Antimicrobial, Antiparkinsonian, Cardiovascular
9	Ar-C(=NN-Het)-N=N-Ar	Antiviral , Anti-inflammatory, Antimicrobial

6.2. Adsorption Activities

Formazan of benzaldehyde (XXV) and formazan of *p*-dimethyl amino benzaldehyde (XXVI) were synthesized by Anand*et al.* (Anand*et al.*, 2009). They were studied as corrosion inhibitor for mild steel in 1.11 N hydrochloric acid by weight loss method. The result showed that the corrosion inhibition efficiency of these compounds was found to vary with the temperature and acid concentration.(XXV) and(XXVI) showed good performance as corrosion inhibitor in HCl solution medium due to the presence of heteroatom and unsaturated bond that cause effective adsorption process leading to the formation of an insoluble protective surface film which suppresses the metal dissolution reaction. Also, it was found that the corrosion inhibition behavior of (XXVI) is greater than that of (XXV). The kinetic treatment of the results gave first order kinetics. The relative corrosion inhibition efficiency of these compounds has been explained on the basis of structure dependent - electron donor properties of the inhibitors.



XXV



XXVI

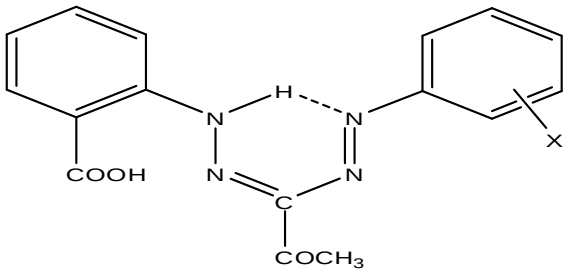
The protection of metal surfaces against corrosion is an important industrial and scientific topic.
Many chemical phenomena cannot be explained by classical physics and need quantum mechanics for the complete analysis. In that case quantum chemical studies are used to analyze the inhibition efficiency of certain compounds on corrosion. Where organic compounds, which can donate electrons to unoccupied d orbital of metal surface to form coordinate covalent bonds and can also accept free electrons from the metal surface by using their anti bonding orbital to form feedback bonds, constitute excellent corrosion inhibitors. Depending on these concepts, Udhayakala*et al.*(Udhayakala*et al.*, 2011), studied the ability of following formazans as corrosion inhibitor:
a)2-(Phenyl (2-phenyl hydrazinyl) methylene) hydrazine carboxamide (XXV)
b)2-((4-(Dimethyl amino) phenyl) (2-phenyl hydrazinyl) methylene) hydrazine carboxamide (XXVI)
And they concluded the following:

1. The inhibition efficiency of formazan derivatives obtained Quantum chemically increase with the increased in E_{HOMO}, and with decreased in E_{LUMO} and energy gap (ΔE). (XXVI)has the highest inhibition efficiency because it had the highest HOMO energy and ΔN values and it was most capable of offering electrons.
2. The parameters like hardness (η), Softness (S), dipole moment (μ), electron affinity (EA), ionization potential (IE), electronegativity(χ) and the fraction of electron transferred (ΔN) confirms the inhibition efficiency in the order of (XXVI) > (XXV).
3. Fukui function shows the nucleophilic and electrophilic attacking sites in the formazan derivatives.A modified electrode, based on electro-deposition of 5,5'-(oxybis(4,1-phenylene))bis(3-(2-hydroxyphenyl)-1phenylformazan onto pencil graphite electrode, was investigated by Gorcay*et al.*for the determination of paracetamol(Gorcay*et al.*, 2014).

6.3. Analytical Activities

Formazan derivatives have achieved considerable attention in analytical chemistry because of their high sensitivity toward many metals and organometals.Formazan compounds having heterocyclic group such as pyridine, thiazole or oxazole ring have been found to be useful photometric reagents with high selectivity and sensitivity(Schurmann, 1996).
The knowledge of p*K*a values provides a basis for understanding the chemical reactions between the compound of interest and its pharmacological target(Schurmann*et al.*, 1998). Additionally, they play a major role in acid-base titrations, complex formation and various analytical procedures. Also, the p*K*a value(s) of a compound influences many characteristics such as its reactivity, spectral properties (colour) and determination of the activity centers of enzymes in biochemistry(Frey *et al.*, 1971).
Formazans are compounds containing the characteristic azohydrazone group (HN=N–CH=N– NH2), which is a good carrier π bonding and chelating properties. In general, the two terminal hydrogen atoms bonded to N atoms can be substituted by aromatic groups and the substituent on the central carbon atom can be varied greatly. Also, formazans behave as very weak acids. NH protons in the structure have partly acidic character properties due to π bonds. The ionization of the NH group is very difficult and the expected pKa value being higher than 15 for 1,3,5-triphenylformazan(Irving *et al.*, 1977).There have been several studies concerning with the pKa values of substituted formazans. Grote *et al*. reported that the p*K*a values of ortho-substituents formazans evaluated in mixtures of dioxane and water were usually higher than 11.00, In addition to the concept of size of the phenyl ring substituents in the *ortho* position (halogen and alkyl groups) as well as the nature of the substituent on the central carbon atom affect the stability and acidity of the formazans. Their tendency to form different isomers influences the coordination chemistry of the ligands and thus their extraction behaviour. Distribution ratios determined for platinum group metals, gold and numerous base metals reveal the selective extractability of Pd (II) from dilute hydrochloric acid. The *ortho*-iodoformazan is the most effective ligand due to its enhanced formazion of a 1:1 Pd (II) complex, whereas all other formazans co-ordinate as 1:2-chelates. Consequently, in the stripping step with an acidified solution of thiourea, the rate of ligand substitution is also comparatively faster in the case of the 1:1-chelate. An effective separation of Pd (II) from an excess of Pt (IV) can be achieved by the iodoformazan(Grote *et al.*, 1987). Lipunova*et al.* studied the electrochemical behavior of some hetarylatedformazans by introducing them into the bulk of carbon containing inks of thik-film screen printed electrodes for determining copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn) (Lipunova*et al.*, 2004).
Several reports on the use of formazans as analytical reagent for the determination of various metal ions have appeared, and the ionization constants of some benzimidazoylformazans were determined spectrophotometrically.Irving and Hutton found that the acidity constant of 3-carboxymethylthio -1,5-diphenylformazan was 12.48. Also, found unusual properties of 3-[1(or 2)-carboxyethyl]thio-1,5-

diphenylformazans are attributed to configurations in which the carboxylic proton is strongly intramolecularly hydrogen-bonded to a quasi-aromatic formazan ring system (Irving and Hutton, 1980). Also, solvent effects on the absorption spectra of some diphenyl and triphenyl formazans in ultraviolet and visible regions were studied. All these studies gave the base to Abdel Ghani*et al.* (Abdel Ghani*et al.*, 1987) for spectrophotometric determination of the acid dissociation constants of some 1,5-diphenyl formazan derivatives (XXVII) in alcoholic-aqueous solutions, and reported that the acid dissociation constants were in the range of 2.30 – 11.75.



XXVII

ABBREVIATIONS

Ar-: Aryl group CASC: Cis-Anti-s-Cis CAST: Cis-Anti-s-Trans CASTC: Cis-Anti-s-Trans-Cis CSSC: Cis-Syn-s-Cis CSSCC: Cis-Syn-s-Cis-Cis CSST: Cis-Syn-s-Trans CSSTC: Cis-Syn-s-Trans-Cis CSSTT: Cis-Syn-s-Trans-Trans ETC: Electron transport chain EtOH: Ethanol DH: Dehydrogenase h: Hour NoxAB DH: Membrane NADH dehydrogenase NoxA and NoxB MeOH: Methanol min: Minuet NBT: Nitroblue tetrazolium salt	PTC: Phase Transfer catalyst Ph-: Phenyl group QDs: Quantum dots MKH_2: Reduced menaquinones R.T.: Room temperature MK: Semiquinones TV: Tetrazolium violet TASC: Trans-Anti-s-Cis TAST: Trans-Anti-s-Trans TASTC: Trans-Anti-s-Trans-Cis TASTT: Trans-Anti-s-Trans-Trans TSSC: Trans-Syn-s-Cis TSSCC: Trans-Syn-s-Cis-Cis TSSCT: Trans-Syn-s-Cis-Trans TSSTC: Trans-Syn-s-Trans-Cis TSSCTT: Trans-Syn-s-Trans-Trans TPF: Triphenyl formazan TTC: Triphenyl tetrazolium salt

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