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Substituent effects on the spectral properties of some 3-substituted formazans

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Abstract

In this study, 3-substitutedphenyl-1.5-diphenylformazans were synthesized with –NO_2 and –COOH groups in the *o*-, *m*- and *p*- positions of the 3-phenyl ring. Their structures were elucidated from elemental analysis, ^{1}H NMR, ^{13}C NMR and IR spectra. The dependence of λ_{max} upon the type of the substituents and their positions on the phenyl ring was investigated using UV–vis spectra. It was observed that there was a shift of λ_{max} towards the blue region with the electron withdrawing –NO_2 and –COOH groups. The size of this chemical shift ($\Delta\lambda_{max}$) was dependent upon the type and position of the substituent on the 3-phenyl ring. A linear relation was obtained between the substituent effect and the Hammett substituent coefficients.

Keywords: Formazans; Spectroscopy; Dyes; Substituent effect

1. Introduction

Since Pechman synthesis of formazans [1], there have been numerous formazans described and their structural features have been evaluated [2–5]. In recent years, crown formazans have become popular [6–8]. Several studies of the synthesis of different complexes [9], their thermogravimetric analysis [10] and their dissociation and stability constants have been reported [11]. Ionization and complexation constants were potantiometricaly and conductometricaly determined [12] and their redox behavior was evaluated [13]. Formazans have important medical applications; the tetrazolium–formazan system is classified as a promoter of vitality [14]. Formazans are used in the Brucella Ring test of milk [15] and the determination of the effects of anti cancer drugs [16,17]. Kebler claimed that this latter application could not be used for every cell [18]. There have also been numerous studies of IR spectra [19], substitent effects on complexation and extraction properties [20] and p*K*a values [21].

Formazans are colored compounds due to π bonds in their structure and they are potential dyes. Unfortunately, they have not gained widespread use in the dye industry. Structural factors which effect their dyeing properties have not been widely studied.

In our previous study [22] we synthesized various 1-substituted formazans and evaluated the effect of substituents on the λ_{max} using UV–vis spectra. A

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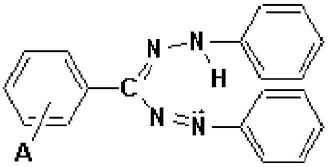


Fig. 1. The structure of formazan derivatives.

similar study was carried out by attaching electron donating substituents to the 3-phenyl ring [23]. The purpose of this present study is to synthesise formazans which have been substituted by attaching electron withdrawing groups at the *ortho*, *meta* and *para* positions of the 3-phenyl ring (Fig. 1) and to investigate the effects of the type and the location of the substituents on absorption λ_{max} values.

2. Result and discussion

This study was carried out in three stages. Initially, the formazans with electron withdrawing groups at each position of the 3-phenyl ring (*o*-, *m*- and *p*-) were synthesized (compounds **1**–**7**). The second stage was the determination of their spectral properties in an investigation of the dependence of their absorption on the substituents. The chemical shift ($\Delta\lambda$) values of the 3-substituted formazans (**2**–**7**) were determined and evaluated. The third stage concerned the determination of the correlation between the effects of the substituents on λ_{max} with Hammett substituent coefficients (σ).

2.1. Synthesis of formazans

The formazans in this study (**1**–**7**) were prepared by the coupling reaction of hydrazones (or substituted hydrazones), with benzendiazonium chloride, in basic medium at 0–5 °C. The hydrazones were obtained by the condensation reaction of aldehydes (or substituted aldehydes) with phenylhydrazine at pH 5–6. While this is a general route in the synthesis of the formazans, it is a highly time consuming, low yield method, which requires both patience and stamina [5]; the purification of the resulting product is also very tedious. The reaction mechanism was followed using UV–vis spectroscopy. The structures of the substituted formazans were elucidated by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The mechanism of the reaction is given in Scheme 1 [5,22]

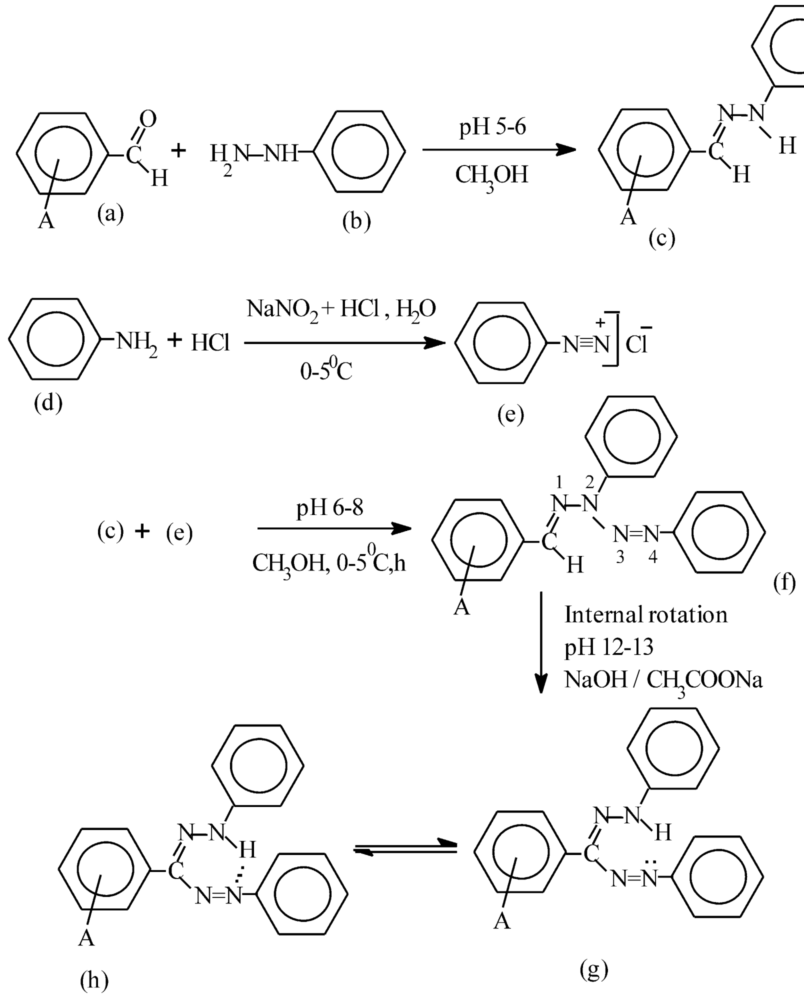
The formation of the intermediate, 1-benzal-(substituted benzal)-2.4-diphenyltetrazene (**f**), was followed using the fact that it has a lighter color than the yellow, orange, or red formazans. Due to the fact that NH proton is more acidic than the CH proton the first coupling of the diazonium salt takes place through the –NH proton [3,5]. However, (**f**) is a very shortlived compound and instantaneously transforms to the formazan (**g**) through intra-molecular rotation in strongly basic media. This conversion was followed by both the change in color and UV–vis spectroscopy. Optimum formazan yield was obtained at a pH value of 12–13. Table 1 lists the features and ^{1}H NMR data of the formazans synthesized.

As seen from Table 1 the yield was higher than those reported in the literature [5,22] due to scrutiny and careful pH adjustment. The reason that the lowest yield was obtained at the *o*-position can be attributed to the substituent being close to the reaction site, which sterically hinders the coupling reaction. The relative increase in yield at both the *m*- and *p*-positions verifies this diagnosis. If an electronic effect were dominant (inductive resonance effect) best yield would be obtained at *o*-position.

The largest difficulty encountered in the synthesis was that the resulting –COOH substituted formazans (**5**–**7**) were plastic-like, resinous and non-crystallizable. The experiments were repeated numerous times using dilute conditions to prevent this situation; however, it was not possible to obtain perfect crystals.

2.2. Spectral properties

Close examination of the ^{1}H NMR values in Table 1 shows that the peaks belonging to aromatic hydrogens at the δ, 8.07–6.70 ppm (15H, *m*) and NH hydrogen at the δ, 1.44 ppm (1H, *s*) for TPF, were found to shift to higher values in the substituted formazans **2**–**7**. In addition, there was a broad signal located at 3.32, 3.40 and 3.34 ppm, corresponding to COOH hydrogen for compounds **5**–**7**. The fact that the signals of the substituted formazans have higher δ values shows that



Scheme 1. The formation mechanism of formazans.

Table 1
Experimental and ^{1}H NMR data of the formazans investigated

Compound	A substituent	Color	Mp °C (lit)	Yield % (lit)	1H NMR data[ab]		
					Aromatic H δ (ppm)	Azo H δ (ppm)	Carboxyl H δ (ppm)
1	H	Cherry red	172–173 (172–174)[22]	70 (71)[22]	8.07–6.70 (15H)*m*	1.14 (1H) *s*	
2	*o*-NO_2	Red-brown	150–152	43	8.34–6.96 (14H)*m*	1.73 (1H) *s*	
3	*m*-NO_2	Red-brown	180–182	62	8.90–6.94 (14H)*m*	1.91 (1H) *s*	
4	*p*-NO_2	Red-brown	196–198 (204–205) [5]	75(51)[5]	8.17–6.83 (14H)*m*	1.96 (1H) *s*	
5	*o*-COOH	Orange	182–183	44	8.44–7.28 (14H)*m*	1.27 (1H) *s*	3.32 (1H) *s*
6	*m*-COOH	Violet	198–200	55	8.69–6.85 (14H)*m*	1.35 (1H) *s*	3.40 (1H) *s*
7	*p*-COOH	Violet	201–202	64	7.95–6.50 (14H)*m*	1.28 (1H) *s*	4.34 (1H) *s*

[a] *m*: multiplet, *s*: singlet.

[b] The ^{1}H NMR spectra were recorded with 400 MHz (in $CDCl_3$).

Table 2
^{13}C NMR data (100 MHz, in $CDCl_3$ and DMSO)

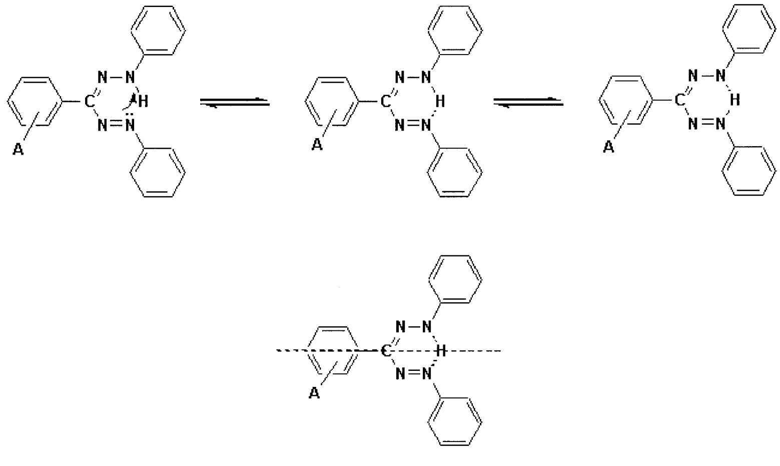
Compound	δ (ppm)
(C=N)	Other carbons
1	148.410, 144.630, 139.871, 134.870, 133.852, 130.632, 128.931, 126.305, 119.303 (Total 9)
2	133.747, 129.532, 129.405, 128.242, 126.484, 126.197, 124.413, 124.169, 121.678, 119.448, 113.503 (Total 11)
3	147.759, 141.391, 133.174, 130.913, 129.888, 128.401, 126.079, 125.167, 121.406, 119.575, 113.898 (Total 11)
4	133.874, 129.926, 129.759, 128.661, 126.197, 124.413, 121.678, 119.443, 113.503 (Total 9)
5	147.094, 142.305, 138.280, 133.550, 132.054, 129.934, 129.184, 127.910, 126.870, 124.038, 121.221, 119.202 (T.12)
6	148.011, 142.407, 138.342, 134.251, 133.241, 130.543, 129.876, 128.203, 127.870, 124.622, 121.203,119.213 (T.12)
7	147.658, 141.471, 136.885, 133.560, 132.124, 129.989, 128.918, 126242, 124.203,119.541 ((Total 10)

the substituent shifts the signals down field. These results can be explained by the fact that the $–NO_2$ and –COOH groups have electron withdrawing ability at every position (*o-, m-, p-)*.

When one examines the ^{13}C NMR data listed in Table 2 there is a 9C signal in contrast to the expected 13C signal for TPF; this shows that TPF is in tautomeric form .The formation of a hydrogen bond between the electron pair on $–N=\ddot{N}–\phi$ and the hydrogen of NH turns the molecule in to a chelate structure which is resposnable for this tautomerism (Scheme 2).

The ^{13}C NMR values of the substituted formazans **2**–**7** show the presence of tautomerism and chelate formation. The fact that one observes an 11C signal instead of a 13C when the $–NO_2$ substituent is located at both the *o*- and *m*-positions (compounds **2** and **3**) and 12C signals instead of 14C when the –COOH substituent is located at the *o*- and *m*-positions (compounds **5** and **6**) can only be explained by a chelate structure. Also, the 9C signals instead of 13C in compound **4** and 10C signals instead of 14C in compound **7** are indications of the symmetry plane of the chelate structure when the substituents are located at the *p*-position [2,24]. The shift down field in the ^{13}C NMR data of the substituted formazans (**2**–**7**) can be related to the electron withdrawing ability of the $–NO_2$ and –COOH groups.

When the IR data of formazans **1**–**7** listed in Table 3 are examined one can see C=N absorption at 1490–1510 cm^{-1}. These values show that the formazans form a chelate structure. As the C=N stretching band appears at 1565–1551 cm^{-1} in the case where there is no chelate structure present (excited state) and 1510–1500 cm^{-1} in the case of chelation [3,19], the synthesized formazans must be in a chelate structure. This agrees with the



Scheme 2. Molecular chelation and symmetry.

Table 3
The IR spectral data of the formazans **1**–**7**, (KBr, cm^{-1})

Compound	O–H group of COOH	Aromatic C–H	N–H	Aromatic C=C	COOH	C=N	$-NO_2$	N=N	CNNC structural vibration
1	–	3069	3050–3000	1600	–	1500	–	1450	930–905
2	–	3250–3100	3050	1600	–	1510	1500–1290	1450	900–800
3	–	3150–3100	3050	1550	–	1490	1520–1320	1360	850–750
4	–	3100–3050	3000–2900	1550	–	1500	1510–1320	1350	850–750
5	3400–3300	3100–3050	3000–2950	1650	1590	1500	–	1350	950–900
6	3390	3050	3050–3000	1550	1600	1500	–	1390	900–800
7	3400–3300	3050	2950–2900	1550	1600	1500	–	1400	900–800

hypothesis that the repulsion of bulky groups such as $-NO_2$ and –COOH, attached to the central 3-phenyl ring, causes the chain structure to convert into a ring form. Other IR data can be evaluated in a similar manner.

2.3. Substituent effects on the UV–vis absorption λ_{max}

Table 4 list all the peaks observed in the UV–vis spectra of the formazan and substituted formazans (**1**–**7**). The chemical shift values ($\Delta\lambda$) were determined by taking the difference between the λ_{max1} values of the substituted formazans with the λ_{max1} value of TPF. Fig. 2 shows the UV–vis spectra of NO_2 formazans (**1**–**4**) for comparative purposes.

The peaks listed as λ_{max1} in Table 4 are the broad peaks which are characteristic of the formazan skeleton (Fig. 2). They are generally observed at 410–500 nm but may shift to 550–600 nm depending upon structure. These peaks are due to π–π* electronic transitions in the formazan skeleton. The sharp λ_{max2} peaks which appear at 300–350 nm correspond to n–π* electronic transitions of the –N=N– group. The λ_{max3} peaks observed at 270–300 nm originate from n–π* transitions of –C=N– groups. These are similar to those corresponding to the –N=N– peaks in appearance. Our discussion will focus on the λ_{max1} values, which are characteristic of the formazan structure.

Table 4
UV–visible absorption maxima of the formazans **1**–**7** (CH_3OH, 10^{-4} mol/l)

Compound	λ_{max1} (nm)	λ_{max2} (nm)	λ_{max3} (nm)	Chemical shift $\Delta\lambda_{max}$	Hammett substituent constant σ
1	483.0	335	298	–	–
2	408.0	342	290	75	–
3	477.0	303	262	6	0.71
4	414.0	256	–	69	0.81
5	429.0	293	219	54	–
6	479.0	337	298	4	0.35
7	467.0	343	230	16	0.44

$\Delta\lambda_{max} = \lambda_{max}1(TPF) - \lambda_{max1}$(substituted formazans).

The λ_{max1} value of TPF at 483 nm, shifts to 408, 477 and 414 nm when the 3-phenyl ring is substituted with a $-NO_2$ group at the *o*-, *m*- and *p*-positions. In all three cases, substitution of the $-NO_2$ group shifts the absorption to lower wavelength (hypsochromic effect). This situation is

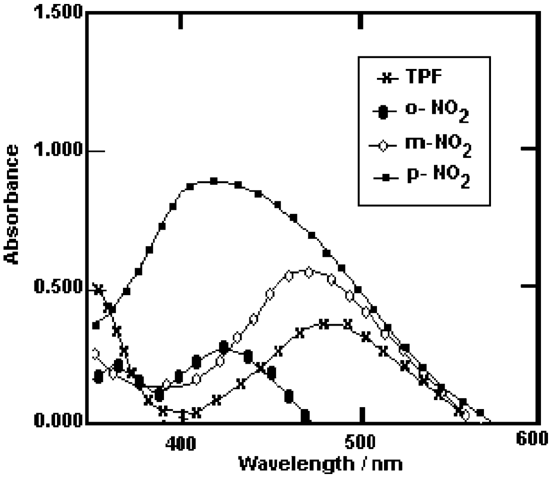


Fig. 2. UV–vis spectra of formazans **1**–**4** (CH_3OH, 10^{-4} mol/l).

compatible with the fact that NO_2 is an electron withdrawing group both inductively and in resonance. The chemical shift values ($\Delta\lambda$) obtained for the *o*-, *m*- and *p*-positions are 75, 6 and 69 nm respectively. The finding that the highest shift was observed in the case of *o*-substitution ($\Delta\lambda$ =75 nm) can be explained by the fact that it is the nearest position to the reaction site and so the NO_2 group inserts the highest inductive effect coupled with the resonance effect; thus, it has the highest electron withdrawing effect. At the *m*-position, however, there is no resonance and the inductive effect is weak; therefore the chemical shift remains merely at $\Delta\lambda = 6$ nm. At the *p*-position, there is a resonance effect but the inductive effect almost disappears due to its large distance from the reaction site. Therefore the chemical shift of $\Delta\lambda = 69$ nm is lower than that obtained for the *o*- position but considerably higher than that given by the *m*-position. This can be attributed to the fact that the resonance effect dominates the inductive effect at the *p*-position.

When the 3-phenyl ring is substituted with a COOH group, the formazan peak observed at 483 nm for TPF shifts to 429, 479 and 467 nm for the *o*-, *m*- and *p*-substitutions respectively. There is again a shift towards the lower wavelength (hypsochromic effect). This can also be explained in terms of the electron withdrawing ability of the COOH group. The highest chemical shift is observed at the *o*-position due to the fact that the COOH group inserts the highest inductive effect in addition to a resonance effect ($\Delta\lambda$ =54 nm). There is only a weak inductive effect in the case of *m*-substitution and so the chemical shift remains at only $\Delta\lambda = 4$ nm. In the *p*-position the inductive effect weakens but there is also a resonance effect. Therefore the chemical shift is higher than that obtained at the *m*-position but it remains lower than that of the *o*-position ($\Delta\lambda$ =16 nm)

Another interesting result is that the $\Delta\lambda$ values for the –COOH substituent are lower for every position than those obtained for the $–NO_2$ group. This can be attributed to the lower electron withdrawing capacity of COOH compared with the $–NO_2$ group. In conclusion, both substituents caused a hypsochromic effect upon the UV–vis spectra of the formazans.

3. Conclusions

3.1. The relation between the $\Delta\lambda_{max}$ values and Hammett substituent coefficients.

Hammett substituent coefficients (σ) are used to evaluate the effects of substituents upon the rate of a chemical reaction for which a mechanism is known. It was decided that we should investigate whether the Hammett substituent coefficients could be used to evaluate the effect of the substituent, on λ_{max} and, thus, the color. For this purpose the λ_{max} values were plotted against the Hammett substituent coefficient σ (Fig. 3).

As seen from Fig. 3 there is a linear relation between the σ and λ_{max} values. Therefore σ values

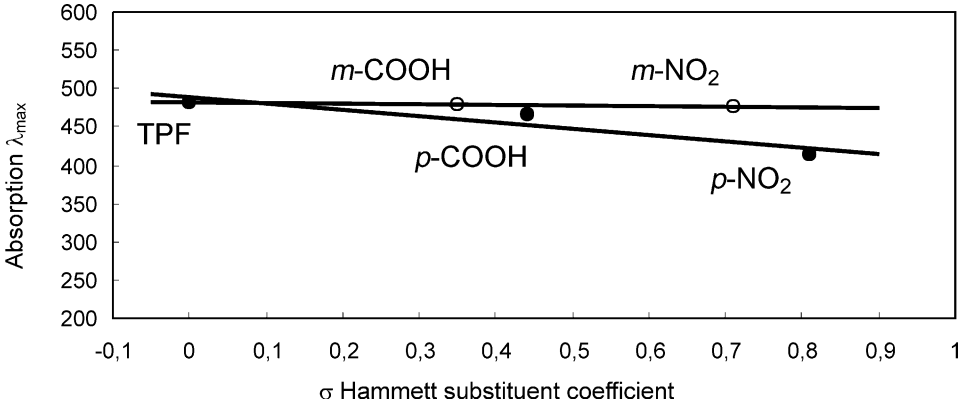


Fig. 3. The plot of Hammett substituent coefficient agains, λ_{max} values for *m*- and *p*-derivatives of formazan.

can be used to evaluate the absorption λ_{max} values. If the σ value is positive (i.e. the substituent is electron withdrawing) then it exerts a hypsochromic effect upon λ_{max}. The amount of shift in the λ_{max} values (i.e. the magnitude of $\Delta\lambda_{max}$) is proportional to the electron withdrawing ability of the substituent depending upon its type and position. The value of $\Delta\lambda_{max}$ is small when only the inductive effect was present. The presence of a resonance effect increases the chemical shift. The highest $\Delta\lambda_{max}$ value is observed when both inductive and resonance effects are operative. In our previous work we emphasized that electron donating groups caused bathochromic shifts in the $\Delta\lambda_{max}$ values [22,23]. Hence, the substitution of electron donating groups in the formazan molecule, increases the conjugation and photosensitization and causes a bathochromic shift upon λ_{max}. On the other hand, the electron withdrawing groups lower the extent of conjugation and thus photosensitization and impart a hypsochromic effect to the λ_{max} values.

4. Experimental

4.1. General

The UV–vis spectra of all the formazans synthesized in this study were obtained with UNİCAM UV2-100 UV/visible spectrophotometer using 1 cm quartz cells in 10^{-4}mol l^{-1} CH_3OH using 325 nm UV lamp in the range of 200–600 nm. The IR spectra were obtained on a MATTSON 100-FT-IR spectrophotometer between 4000 and 400 cm^{-1} using KBr pellets. ^1H NMR spectral studies were performed on a Brüker 400 MHz and ^{13}C NMR 100 MHz spectrophotometer using $CDCl_3$, 10^{-4} mol l^{-1}. All the elemental analysis studies were carried out using a LECO-CHNS-932 elemental analyser.

4.2. General synthesis

Syntheses were carried out using benzaldehyde (or substituted benzaldehydes), phenylhydrazine and aniline. Benzaldehyde (or substituted benzaldehydes) were reacted with phenylhydrazine at pH 5–6 to obtain benzaldehyde phenylhydrazone (or substituted benzaldehyde phenylhydrazones). These hydrazones were then coupled with benzene diazonium chloride at 0–5 °C.

4.2.1. Synthesis of 1.3.5-triphenylformazan (1)

Benzaldehyde phenylhydrazone was synthesized by the reaction of benzaldehyde (2.12 g, 0.02 mol) with phenylhydrazine (2.16 g, 0.02 mol) in a methanolic medium at pH 5–6. It was dissolved in an appropriate amount of methanol and mixed with buffer [sodium hydroxide (2.50 g), sodium acetate (3.50 g) and methanol (200 ml)]. In another flask the diazonium chloride solution was prepared using aniline (1.86 g, 0.02 mol) concentrated HCl (5 ml) and sodium nitrite (1.50 g) at 0–5 °C. Benzenediazonium chloride solution was added to the buffered benzaldehyde phenylhydrazone solution dropwise with constant stirring. The mixture was stirred for 2 h and the ensuing bright red colored formazan was kept in a fridge for 2 days and recrystallized from methanol.

4.2.2. Synthesis of 3- (o-, m-, p-nitrophenyl)-1,5-diphenylformazans (2-4)

o-, m- And *p*-nitrobenzaldehyde (3.02 g, 0.02 mol) were dissolved in methanol (25 ml) and phenylhydrazine (2.16 g, 0.02 mol) was gradually added to with constant stirring at pH 5–6 . The procedure was completed in 30 min. The resulting blood-red hydrazone was left on the bench over night and was then filtered and recrystallized from methanol. The *o-, m-* and *p*-nitrobenzaldehyde phenylhydrazone (2.41 g, 0.01 mol) was dissolved in methanol (200 ml) by constant stirring under reflux and buffer solution (prepared as before). In another flask benzendiazonium chloride solution was prepared using aniline (0.93 g, 0.01 mol) concentrated HCl (5 ml) and sodium nitrite (0.75 g) at 0–5 °C. This solution was added to the *o-, m-, p*-nitrobenzaldehyde phenylhydrazone solutions drop-wise with constant stirring to form compounds **2**–**4**. The solution was stirred for 2 h at the same temperature and kept in a cupboard for 2 days. Each compound was recrystallised from methanol. Elemental Analysis for $C_{19}H_{15}N_5O_2$, M: 345. Calc. (%): C, 66.08; H, 4.34; N, 20.28; O, 9.27. Found: (%) C, 65.98; H, 4.27; N, 19.98; O, 9.36.

*4.2.3. Synthesis of 3-(o-, m-, p-carboxyphenyl)-1,5-diphenylformazans (**5-7**).*

The procedure was the same as earlier except that acetic acid was added to the medium to precipitate the products which were kept in a cupboard for 5 days. The ensuing materials were gum-like and resinous. The experiments were repeated several times in very dilute media and at varied pH but, again, resinous products were obtained. This was attributed to the polymerization of the resulting formazans. Elemental Analysis for $C_{20}H_{16}N_4O_2$, M: 344. Calc. (%): C, 69.76; H, 4.65; N, 16.27; O, 9.30. Found: (%) C: 69.01; H: 4.15; N: 15.98; O: 9.37.

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