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THE COMPARATIVE STUDY OF THE INFLUENCES OF THE EXTERNAL FACTORS ON THE UV-VIS ABSORPTION SPECTRA OF SOME POTENTIALLY TAUTOMERIC AZOCOUPLING PRODUCTS

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ABSTRACT. The influences of the pH', water content, sample concentration, temperature and time on the UV-Vis absorption spectra of four potentially tautomeric azocoupling products **1**- **4** have been comparatively studied in ethanol-water mixtures. The pH' influence can be explained by the involvement in two steps deprotonation equilibria of each dye **1**- **4**. These equilibria are characterized by the $pK_a^{'}$ values. The three species involved in the two deprotonation steps of each dye **1**- **4** exhibit different visible absorption maxima. The hydrazone structures **1b**- **4b** of the initial neutral species of the dyes are supported by IR and ^{1}H-NMR data. The presence of other tautomeric forms (*e.g.* **1a- 4a**) has been not proved. The comparative study of the effect of other external factors on the spectra with that of pH' has shown that: i)- the influence of either water content, sample concentration or temperature, which is characterized by the presence of isosbestic points, may be explained by the involvement of the dyes in the first step acid ionization; ii)- the influence of either water content, sample concentration, temperature or time, when isosbestic points do not appear, might be explained by the superposition of the acid ionization equilibrium of the dyes with other phenomena. Thus, to account for the effect of water content and time on the spectra of the dye **1,** the superposition of a disaggregation process and acid ionization equilibrium has been assumed.

Keywords: *azacoupling products, UV-Vis spectra of azacoupling products*

INTRODUCTION

The azocoupling products having hydroxyl groups conjugated with the azo linkage from their potentially azo tautomer might be involved in azo-hydrazone [1a, 2-11], acid-base [1a, 2, 8, 9, 11-19] or/and aggregation [1b, 3, 9-11, 14, 20-30] equilibria. All these equilibria are influenced by the structure

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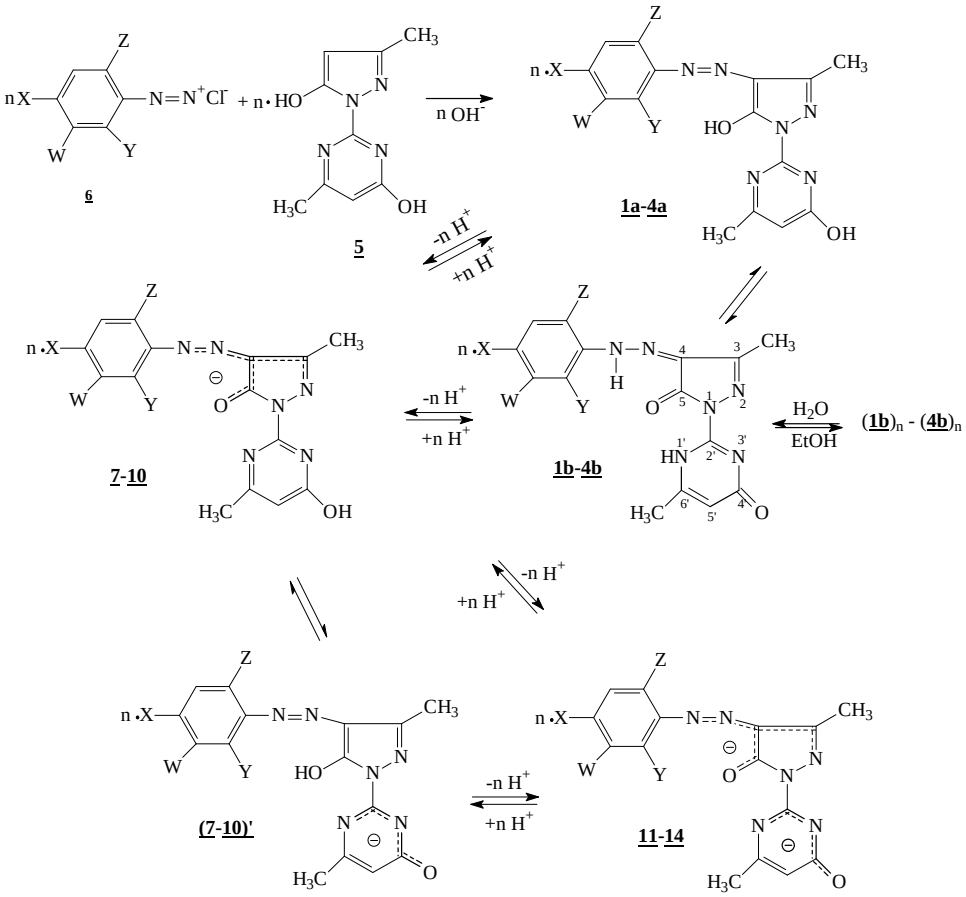
of the azocoupling products [1a, 2, 8, 9, 11-13, 16-19, 22, 27, 28, 31-34] and by external factors [1a-c, 2, 3, 6, 8-28, 30-45]. Because the forms involved in the possible equilibria have different chemical structures they might differ also in colour [1a, 1c, 2, 3, 12a, 30]. Therefore the factors that influence the equilibria might change the colour. Such a colour change has consequence on the application possibilities of the azocoupling products [1a, 1c, 2]. Therefore the study of the influence of different factors on the UV-Vis absorption spectra of the azocoupling products has a practical significance [1a-c, 2, 12a, 31, 32, 37-39]. On the other hand, it has been studied the influences of the various factors on the UV-Vis spectra of the azocoupling products in order to establish the nature of the equilibria exhibited by dyes [1b, 2, 3, 6, 8-26, 28-37, 40-45]. In a study using UV-Vis spectrophotometry, the certain criterion for the existence of an equilibrium is the occurrence of isosbestic points on the recorded spectra under various values of investigated factor [1b, 2, 8, 9, 11-14, 17-19, 22-26, 41-45]. In exchange, the criterion to find out the nature of the equilibrium involved is more or less certain. Thus, the criterion for the acid-base nature of the equilibrium is the occurrence of isosbestic points on the UV-Vis absorption spectra recorded with several isomolar solutions of the dye at different pH values [9-14, 17-19, 25, 28, 41, 42]. The certainty of acid- base nature of the equilibrium relies on sigmoidal shape of the plot of absorbance at analytical wavelength against pH [1b, 9, 17, 18, 41, 42]. The criterion for the azo-hydrazone nature of the equilibrium is usually considered the presence of isosbestic points on the superposed spectra of isomolar solutions of azocoupling product in binary mixtures of the same solvents with different mixture composition [2, 18, 45]. Also, as a rule, the criterion for aggregation nature of the equilibrium is the occurrence of isosbestic points on the spectra obtained at different dye concentrations but keeping a constant value of $C_{Di} \cdot l_i$ [1b, 23, 24, 26, 28b].C_D stands for molar concentration of dye and *l* for optical path length of the cell. However, the presence of isosbestic points as the solvent or concentration of the azocoupling products is changed was interpreted by other authors by involvement of acid-base equilibrium [8, 9, 12b, 13]. Contradictory interpretation was also given in the analysis of temperature [3, 9b, 12b, 13, 18, 22, 24, 29, 32, 35] or time [9, 10, 20, 30] dependence of the spectra. The nature of the exhibited equilibrium is even more difficult to ascertain for azocoupling products that may be involved in several types of equilibria [8-10, 14, 22-25]. Our investigating products **1-4** belong to this category. Therefore, the aim of this work is to evaluate the criteria of the UV-Vis spectrophotometry for establishment the nature of the equilibria exhibited under the influence of various factors by each azocoupling products **1-4**. With this aim in view a comparative study of the effects of various factors on the spectra of azocoupling products has been undertaken using ethanol- water mixtures. Spectra were recorded at

several pH values (Fig 1, 2a, 3, 4a, 5a, 6), water content (Figure 2b, 5c, 7), sample concentration (Figure 4b, 5b, 8c, 9), temperature (Figure 8b, 10) and various periods of time after the preparation of solutions (Figure 11a, b). Also an absorption spectrum in solid state (Figure 12) was recorded. The products **1**-**4** belong to the 4-arylhydrazono-3-methyl-1-(6-methyl-5R-4-X-pyrimidin-2-yl)-pyrazolin-5-ones, that are studied by our group for some time [6, 9, 33, 40-42, 46, 47]. The dyes **1**-**4** were obtained from 1-(4-hydroxy-6- methylpyrimidin-2-yl) pyrazolin 5-one (**5)** and nitrobenzenediazonium salts (**6)** (Scheme 1)



1; **7**; **11**; X =NO_2; Y=Z=W=H
2; **8**; **12**; X =NO_2; Y=Z=Cl; W=H
3; **9**; **13**; X=Z=W=H; Y=NO_2
4;**10**; **14**; X=Y=Z=H; W=NO_2

Scheme 1

RESULTS AND DISCUSSION

Preliminary remarks

The azocoupling products **1**-**4** may exist potentially in several tautomeric forms (*e.g.* **1a**-**4a**; **1b**-**4b** Scheme 1) [33, 40-42, 46, 47]. Since these tautomeric forms have two mobile hydrogen atoms (HO- or NH-), they may be involved [41, 42] in two-steps ionization equilibria to form **7**-**10** (**7**-**10**)' and **11**-**14** respectively [9, 14, 19]. Also, similar to other azocoupling products [1b, 3, 10, 14, 20-30], the dyes **1**-**4** might be involved in aggregation equilibria to form $(\mathbf{1b})_n$-$(\mathbf{4b})_n$. In spite of all these, UV-Vis absorption spectra in some aprotic solvents as benzene, chloroform, dimethyl sulfoxid, as well as IR spectra in solid state and the NMR-spectra in $CDCl_3$ (Table 1), have detected the presence of a single chemical species [33, 40-42] for each dye **1**-**4**. The same result was also found in the case of other similar pyrazolin-5-one azocoupling products [2, 4-6, 9, 33, 40, 41]. The structure of this species has been assigned to the hydrazone tautomer (*e.g.* **1b**-**4b**), especially on the basis of NMR data [2, 4-6, 9, 33, 40-42]. The undoubted proof for this form is considered the appearance on the ^{1}H-NMR spectra of the pyrazolin-5-one azocoupling products of a singlet signal in the range 13-15 ppm (cf. Table 1), that has been demonstrated to be characteristic for the hydrazone-hydrogen atom (-HN-N=C<) from such compounds [2, 4-6, 9, 33, 40, 41].

Table 1. Some I.R. and ^{1}H-RMN data of the azocoupling products **1**-**4**.

Dye	I.R. data (υ [cm^{-1}] in KBr pellets				^{1}H-RMN data (δ[ppm]) in $CDCl_3$		
	$\upsilon_{C=O}$ (pyrazolin-5-one)	$\upsilon_{C=O}$ (pyrimidin-4'-one)	υ_{NO2} antisym	υ_{NO2} sym	δ $H_{5'}$ singlet	δ NH or OH (pyrimidin-4'-one) singlet	δ NH (hydrazone) singlet
1	1696	1673	1556	1344	6.15	10.93	13.17
2	1690	1680	1556	1340	6.15	10.73	13.19
3	1711	1674	1543	1333	6.14	11.15	14.63
4	1697	1672	1535	1352	6.04	11.0	13.09

However, the UV-Vis absorption spectra of the dyes **1**-**4** in other solvents, particularly in ethanol –water or methanol solutions by the increase of acid, base or water content (Figure 2a, 3, 5a, 6), are compatible with the existence of an equilibrium between at least two species, one of them has been established to be the hydrazone tautomer **1b**-**4b** [33, 40-42].

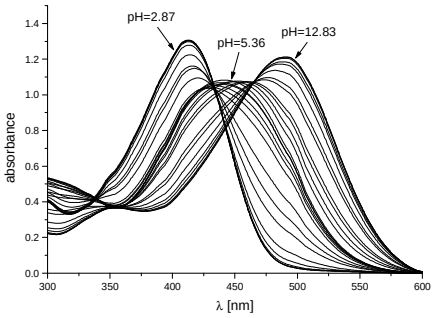


Figure 1. The pH' dependence of the UV-VIS absorption spectra of the isomolar solutions of **1** ($2.25 \cdot 10^{-5}$ mol·L^{-1}, 5 cm cells) in the pH' range 2.87-12.8.

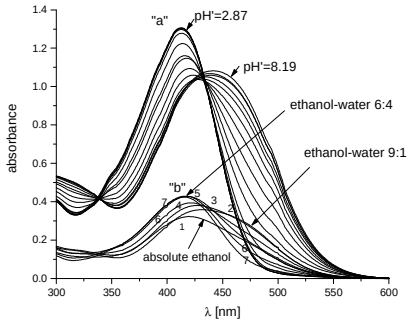


Figure 2. a) The pH' dependence of the UV-VIS absorption spectra of the isomolar solutions of **1** ($2.25 \cdot 10^{-5}$ mol·L^{-1}, 5 cm cells) in the pH' range 2.87-8.19.
b) The water content dependence of the UV-VIS spectra of the isomolar solutions of **1** ($2.68 \cdot 10^{-5}$ mol·L^{-1}, 2 cm cells) in ethanol/ water mixtures **1**-10v/0v; **2**- 9v/1v; **3**-8v/2v; **4-**7v/3v; **5**-6v/4v; **6**-5v/5v; **7**-4v/6v.

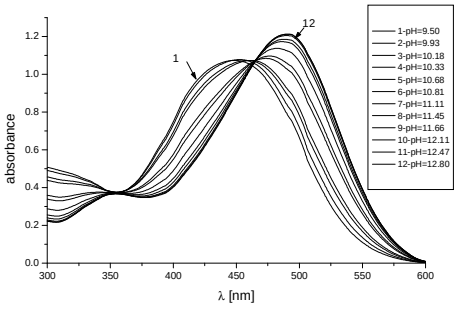


Figure 3. The pH' dependence of the UV-VIS spectra of the isomolar solutions of **1** ($2.25 \cdot 10^{-5}$ mol·L^{-1},5 cm cells) in the pH' range 9.5-12.8.

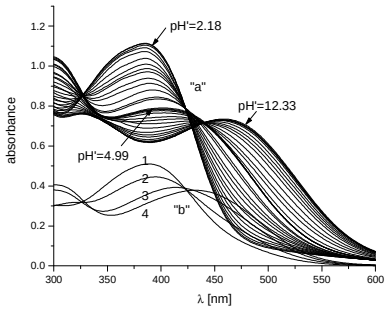


Figure 4. a) The pH' dependence of the UV-VIS spectra of the isomolar solutions of **2** ($2.35 \cdot 10^{-5}$ mol·L^{-1}, 2 cm cells) in the pH' range 2.18-12.33. b) The concentration dependence of the UV-Vis spectra of **2** at 25^0C. The dye concentration and path length of the cell are given: **1)**-$7.06 \cdot 10^{-5}$; 0.5, **2)**-$3.53 \cdot 10^{-5}$; 1, **3)**-$1.76 \cdot 10^{-5}$; 2, **4)**-$7.07 \cdot 10^{-6}$; 5.

Similar behaviour of other azocoupling products have been considered to indicate azo-hydrazone- [2, 6, 18, 33, 40, 43-45], acid-base- [9, 11, 12, 16, 17, 42] or aggregation- [3, 30] equilibria, possible interconnected [8, 13, 14]. Therefore in the next sections we try to establish which of the equilibria in Scheme 1 are in fact involved.

The pH' influence. Determination of the pK_a' values

Since the azocoupling products **1**-**4** are insoluble in water the pH influence on the UV-Vis absorption spectra of these was investigated in ethanol-water (1v/1v) solutions. For this reason we use the notations pH' and pK_a'.

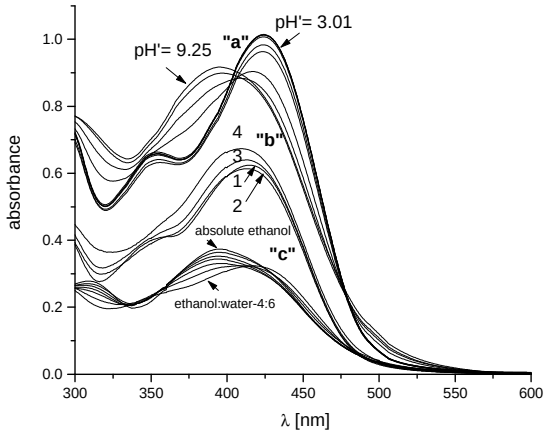


Figure 5. a) The pH' dependence of the UV-VIS spectra of the isomolar solutions of **3** ($3.52\cdot10^{-5}$ mol·L^{-1}, 2 cm cells) in the pH' range 3.01-9.25. b) The concentration dependence of the UV-Vis spectra of **3** at 25^{0}C. The dye concentration and the path length of the cells are given: **1)**-$9.0\cdot10^{-5}$; 0.5, **2)**-$4.5\cdot10^{-5}$; 1, **3)**-$2.25\cdot10^{-5}$; 2, **4)**-$9.0\cdot10^{-6}$; 5. c) The water content dependence of the UV-VIS spectra of **3** ($1.22\cdot10^{-5}$ mol·L^{-1}, 2 cm cells) in ethanol-water mixtures. **1)**-absolute ethanol; **2)**-ethanol-water (9v/1v); **3)**-ethanol-water (8v/2v); **4)**-ethanol-water (7v/3v); **5)**-ethanol-water (6v/4v); **6)-**ethanol-water (5v/5v); **7)**-ethanol-water (4v/6v) (2 cm path length cell).

The different pH' values in the range 2-13 were obtained by addition of either HCl or KOH- solution, having the concentration 10^{-2}-10^{-1}mol·L^{-1}, to discourage dye aggregation [9, 11]. As can be observed in Figure 1, 2a, 3, 4a, 5a, 6, the spectra recorded at different pH' values differ usually both by the position (λ_{max}) and intensity (ε_{max}) of the longest wavelength absorption maximum. Regards the spectra of the isomolar dye solutions over the whole range of pH', one can observe that isosbestic points do not appear

with all these recordings (Figure1, 4a). On the other hand, if the solution pH' is modified either in alkaline (Figure 3), or acid to weak alkaline (Figure 2a, 5a, 6) domain, spectra corresponding to each of the two pH' regions exhibit isosbestic points.

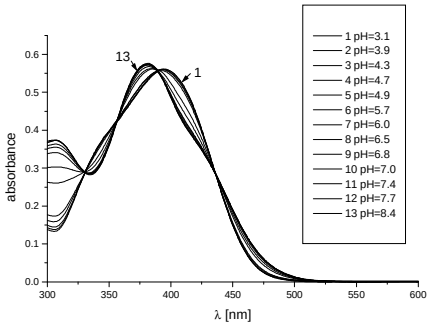


Figure 6. The pH' dependence of the UV-VIS absorption spectra of the isomolar solutions of **4** ($3.66 \cdot 10^{-5}$ mol·L^{-1}) in the pH' range 3.1-8.4.

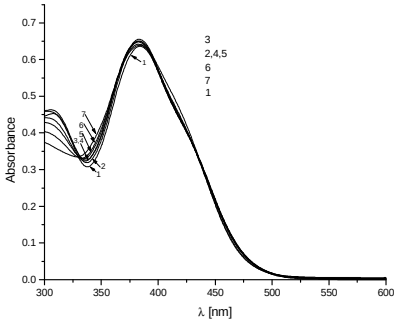


Figure 7. The water content dependence of the UV-VIS absorption spectra of the isomolar solutions of **4** ($c = 2.44 \cdot 10^{-5}$ mol·L^{-1}) in ethanol: water mixtures:**1)**- 10v/0v; **2)**-9v/1v; **3)**-8v/2v; **4)**-7v/3v; **5)**-6v/4v; **6)-**5v/5v; **7)**-4v/6v.

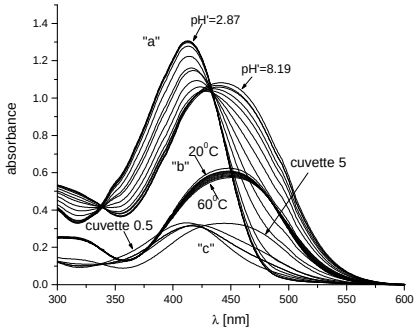


Figure 8. a) Identical with Figure 2a; b) The temperature dependence of the UV-VIS spectra of **1** ($c = 3.09 \cdot 10^{-5}$ mol·L^{-1}) The concentration dependence of the UV-Vis spectra of **1** at 25^{0}C. c) The dye concentration and the length of the cells are given. **1)**-$3.09 \cdot 10^{-5}$; 0.5, **2)**-$1.54 \cdot 10^{-5}$; 1, **3)**-$7.7 \cdot 10^{-6}$; 2, **4)**-$3.09 \cdot 10^{-6}$; 5.

For example, in the case of the dye **1** the two pH' range, in which the spectra exhibit isosbestic points, are: 9.5 - 12.83 (Figure 3), and 2.87 - 8.19 (Figure 2a), respectively. By plotting the peak absorbance *vs*. pH' for each of the two pH' regions a sigmoidal curve has resulted, as exemplified by Figure 13 for the pH' region 2.87- 8.19 in case of dye **1**. The two sigmoidal curves obtained for each dye prove the involvement of the dyes into two acid-base equilibria that account for the pH' influence on the UV-Vis absorption

spectra. Based on the fact that the compounds **1**-**4** are potentially AH_2 acids, the above described behaviour should correspond to the two-step acid ionization equilibria of the type: $AH_2 \leftrightarrows AH^- + H^+$ and $AH^- \leftrightarrows A^{2-} + H^+$. The species: AH_2, AH^-, A^{2-} involved could be identified by characteristic visible absorption bands for each dye. The position and the intensity of the corresponding maxima are presented in Table 2. For example, in the case of dye **1** these maxima have λ_{max} at 412.5 (AH_2 = **1b**), 441.5 (AH^- = **7** or **7'**) and 490.5 nm (A^{2-} = **11**), respectively (Figure 1). The structures of the unionized species AH_2, for the dyes **1**-**4**, correspond to the hydrazone tautomers **1b**-**4b,** while the ionized species AH^- and A^{2-}, as for other azocoupling products [2, 9, 11, 12, 28b, 33, 40-42], should have structures that are predominantly azo in character, corresponding to **7**-**10** or (**7**-**10**)' for the anion (AH^-), and to **11**-**14** for the anion (A^{2-}) (Scheme 1).

Table 2. The UV-VIS spectra data {λ_{max}[nm]; (ε_{max} [L mol^{-1} cm^{-1}])} and the denotation of the species (of type AH_2, AH^-, A^{2-}) involved in the deprotonation equilibria of the azocoupling products **1**-**4** and the corresponding pK_a' values, in ethanol-water (1v/1v).

Dye	The type of species involved			the pK_a' values	
	AH_2	AH^-	A^{2-}	pK_{a1}'	pK_{a2}'
1	412.5 (29038) pH′=2.87 **1b**	441.5 (24046) pH′=8.19 **7**	490.5 (26928) pH′=12.8 **11**	5.84	10.56
2	386 (23705) pH′=2.18 **2b**	415 (16121) pH′=6.56 **8**	461 (15482) pH′=12.4 **12**	3.87	10.34
3	424 (14408) pH′=3.01 **3b**	394.5 (13027) pH′=9.25 **9**	430 (12551) pH′=12.74 **13**	6.88	10.88
4	394 (30638) pH′=3.1 **4b**	381.5 (31378) pH′=8.4 **10**	397 (27034) pH′=12.5 **14**	5.63	11.0

Moreover the sigmoidal curves enable us to determine the pK_a' values of the two-step ionization equilibria of the dyes by means of the inflection points. These are precisely determined by means of the first derivative of each curve as shown in Figure 14 [9, 41,42]. The pK_{a1}' and pK_{a2}' values for each dye **1**-**4** are presented in Table 2.

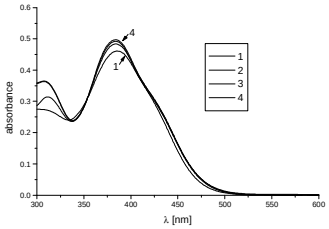


Figure 9. The concentration dependence of the UV-VIS spectra of **4** at 25^0C. The dye concentration and the path length of the cells in cm are given. **1)**-$1.07\cdot10^{-4}$; 0.5, **2)**-$5.35\cdot10^{-5}$; 1, **3)**-$2.67\cdot10^{-5}$; 2, **4)**-$1.07\cdot10^{-5}$; 5.

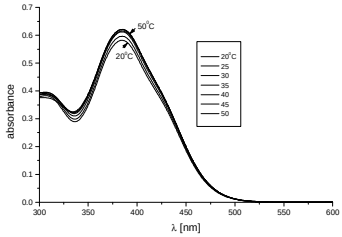


Figure 10. The temperature dependence of the UV-VIS spectra of **4** ($5.35\cdot10^{-5}$ mol·L^{-1}).

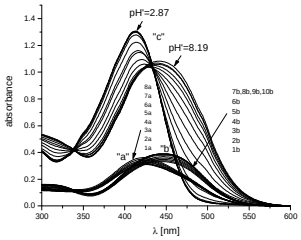


Figure 11. a) The time dependence of the UV-VIS spectra of **1** ($2.81\cdot10^{-5}$ mol·L^{-1}, 1 cm cells) in absolute ethanol (Chimopar). The time delay in minutes. **1)**-0 ; **2)**-1; **3)**-6; **4)**-24; **5)**-36; **6)**-79; **7)**-168; **8)**-255. **b)** The time dependence of the UV-VIS spectra of **1** ($2.11\cdot10^{-5}$ mol·L^{-1}, 1 cm cells) in absolute ethanol (Chimopar: Cristal 3v/1v).). The time delay in hours. **1)**-0; **2)**-0.016; **3)**-0.083; **4)**-0.116; **5)**-0.3; **6)**-1; **7)**-1.83; **8)**-3.56; **9)**-24; **10)**-48; **11)**-72. (1 cm path length cell). **C)** Identical with Figure 2a.

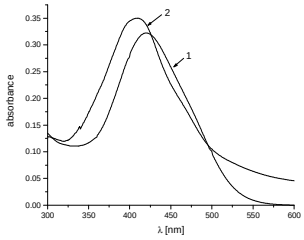


Figure 12. The UV-VIS absorption spectra of **1. 1)** in absolute ethanol solution; **2)** in solid state.

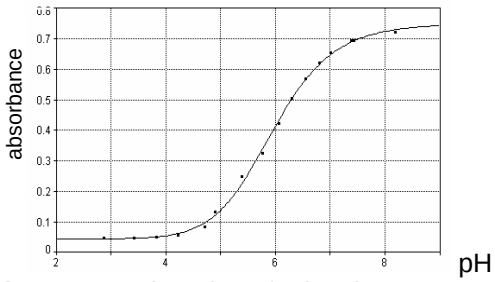


Figure 13. The plot of absorbance *vs.* pH` corresponding to the absorption curves from Figure 2a, at analytical wavelength of 490 nm.

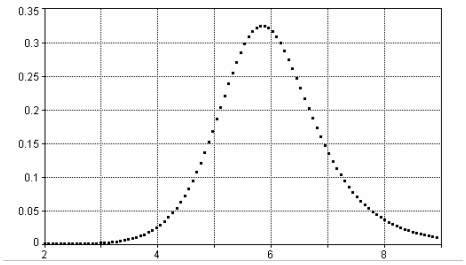


Figure 14. The first derivative of the plot from Figure13, that yields the pK_{a1}' value for **1**.

Interesting structural effects were observed from the manner of pH' influence on the spectra. The increase of pH' in the range of first step ionization causes a bathochromic shift of the longest wavelength band in the case of azocoupling products **1**, **2** (Figure 2a; 4a) having the nitro group in *para* position on their benzene ring relative to N-N bridge, even if there are other subtituents present[9a]. On the contrary, the same pH' increase determines a hypsochromic shift of the maximum in the case of the azocoupling products having the nitro group in *ortho* (**3**) (Figure 5a) or *meta* (**4**) (Figure 6) position. It should be noted that this last behaviour is similar to that observed in the case of other azocoupling products that do not have nitro group, obtained also from **5** or its derivatives [6, 9, 33, 40, 41]. The exception caused by the nitro group in *para*-position in the manner of pH' influence on spectra is probably determined by the possibility of a very strong conjugation between this group and the negatively charged oxygen atom bound in position 5 of the pyrazole ring of the corresponding de-protonated species **7**, **8** [40]. Such strong conjugation is favoured by the known planarisation effect caused by a nitro group located at one end of an extended delocalized π-electron system through which it is able to conjugate directly with a strong electron donating group from the other end of the mentioned system [48].

Concerning the pKa values, it is worth mentioning that pKa_1' values are much more influenced by the nature and position of the substituent as compared to the pKa_2' values. The pKa_1' value of *ortho* substituted derivative (**3**) is greater than the pKa_1' value of the corresponding *meta*- (**4**) or *para*- (**1**) substituted-derivative as found with other azocoupling products [1a,11,12a] (cf. Table 2). The *ortho*-effect [40] accounts for larger pKa_1' value of the *ortho* substituted derivative.

The influence of water content in ethanol - water mixtures

The UV-Vis absorption spectra of the isomolar solutions of the dyes **1**-**4** in ethanol - water mixtures depend on the water content (Figure 2b, 5c, 7) in a different manner. Thus, the spectra of dyes **1**, **2** recorded at different water content in ethanol - water mixtures, do not exhibit isosbestic points (Figure 2b). The corresponding spectra of **3** and **4** (Figure 5c, 7) exhibit isosbestic points even if these are more or less distorted. Therefore, it may be admitted that water content dependence of the spectra of the dyes **3** and **4** indicates the presence of an equilibrium. The similarity of this dependence with the pH' dependence corresponding to the first ionisation step (Figure 5a, 6) infers that water content effect implies the same type of ionization equilibrium, namely **3b⇆9** and **4b⇆10** respectively. A similar reasoning was given also by other authors for the solvent effect in the case of other azocoupling products [9, 12b, 13, 41]. In accordance with the above assumption, the progressive increase of water content of the mixture (Figure 5c) has a similar effect on

the spectra as that of acidity increase in the range of first acid ionisation of **3** (Figure 5a) It consists in a bathochromic shift, corresponding to the change of equilibrium **3b⇆9** towards hydrazone form **3b**, that has λ_{max} at 424 nm, while the anion **9** has λ_{max} at 394.5 nm. This shift caused by the increase in water content of solution is in agreement with the fact that the water is a stronger acid than ethanol[9, 13]. It is however worth mentioning that such a shift of the observed equilibrium in the case of dye **3** towards **3b** by the water content increase is compatible with azo-hydrazone nature of this equilibrium, taking into account the assessment that water displaces the azo-hydrazone equilibrium towards hydrazone tautomer [2, 28, 43-45]. Nevertheless, because the presence of the azo tautomer **3a** is not proved, the probability that water content dependence observed in Figure 5c to be caused by azo-hydrazone equilibrium is correspondingly very low. But the possibility to explain the observed effect of the water content either on the basis of ionization or azo-hydrazone equilibrium shows that the usual criterion to assess the azo-hydrazone nature of the equilibrium exhibited by a potentially tautomeric azocoupling product is somehow in doubt.

On the other hand, the appearance of distorted isosbestic points, when water content effect on the spectra of the dye **3** (Figure 5c) was examined, may be caused by the superposition of the acid-base equilibrium with other phenomena, including other equilibria [9, 14]. Such a superposition rather occurs with the dyes **1**, **2**, where water content dependence of the spectra does not exhibit isosbestic points (Figure 2b). The last assumption is confirmed by a comparative study of the water content dependence of dye **1** spectra with the corresponding pH' dependence in the range of first acid ionization (pKa_1', Figure 2a). The simple comparison of the Figure 2a and 2b shows that apparently the species involved in both dependences are the same. This situation is compatible with the possibility that also for the dye **1** one of the reason of the water content dependence of the spectra to be an acid- base equilibrium. A strong support for that is the hypsochromic shift observed by the stepwise increase of water content of solutions of the dye **1** in ethanol- water mixtures from the composition 9v/1v (curve 2 in Figure 2b) up to composition 4v/6v (curve 7). This shift is in perfect agreement with the similar shift caused by the increase of acidity in the range of first acid ionisation (**1b⇆7**, Fig, 2a). In fact, the discussed hypsochromic shift is brought about by the protonation of the HA^- species **7** (λ_{max} = 441.5 nm), to the neutral hydrazone H_2A species **1b** (λ_{max} = 412.5 nm). However, similar to the dye **3,** the water content dependence of the spectra illustrated by the curves 2-7 from figure 2b is also compatible with azo-hydrazone equilibrium. But the probability that the water content dependence illustrated by curves 2-7, to be mainly determined by the azo-hydrazone equilibrium is very low, since the presence of the azo tautomer **1a** is not proved.

On the contrary, the bathochromic shift noticed when compare the spectrum of dye **1** in absolute ethanol with that of the ethanol-water solution in the ratio 9v/1v, cannot be rationalized only on basis of the involvement of an acid-base equilibrium. As shown above, the water addition should cause a hypsochromic shift (Figure 2b). Also, the bathochromic shift observed by the passing from curve 1 (λ_{max} at 420 nm) to curve 2 (λ_{max} at 435 nm) is not compatible with azo-hydrazone equilibrium, because it is considered that water shifts equilibrium towards hydrazone form [2, 28, 43-45]. This form of dye **1** exhibits an absorption maximum located at 412.5 nm (a hypsochromic shift). Consequently the bathochromic shift found should be caused by the involvement of other phenomena along with acid-base equilibrium by changing the water content. Such a phenomenon might be a disaggregating process of an aggregate $(\mathbf{1b})_n$ formed from molecules of hydrazone **1b**. Such an aggregate is expected to have the visible absorption band superposed on that of the hydrazone **1b,** as occurs with other aggregates of azocoupling products [21,23,24].

The aggregate is somehow stable in absolute ethanol. It disintegrates easily at water addition. Further, the hydrazone **1b** is involved simultaneously with its formation from the aggregate, in ionization equilibrium **1b** ⇆ **7** (Scheme 1). The position of this equilibrium in the ethanol-water (9v/1v) solution correspond to a partial ionization to **7** (100% **7** has λ_{max} = 441.5 nm, Table 2). Thus, the bathochromic shift observed comparing curves 1 and 2 (Figure2b) is in agreement with a partial disaggregating process $(\mathbf{1b})_n \rightarrow n\ \mathbf{1b}$, accompanied by the establishing the acid-base equilibrium **1b** ⇆ **7**. Due to the presence of the anion **7** in this solution, the absorption maximum is bathochromically shifted relative to the absorption maximum of the aggregate and of the hydrazone form **1b** (~ 412 nm). The possible existence of the aggregate $(\mathbf{1b})_n$ is also argued in Sections 3.6 and 3.7.

The influence of the sample concentration

The UV-Vis absorption spectra of the dyes **1**-**4** in ethanol solutions, modifying the dye concentration in the range 10^{-6} - 10^{-4}mol·L^{-1} and keeping constant the product $c_D^i \cdot l^i$, exhibit isosbestic points in most cases (Figure 4b; 8c; 9), even if some are distorted (Figure 4b, 8c). An exception was found where isosbestic points do not appear (Figure 5b). When isosbestic points appear (dyes **1**, **2**, and **4**) by concentration change, an equilibrium should be present. The decrease of the dye concentration has an effect on UV-Vis absorption spectra (Figure 4b, 8c, 9) similar to the effect caused by the increase of the pH' in the region of first acid ionization step (Figure 4a, 8a, 6). Based on these findings the most reasonable cause for the dye concentration effect in the case of dyes **1**, **2** or **4** should be the equilibrium corresponding to the first acid ionization step. This is supported by the known fact that the

ionization of an acid (e.g. $AH_2 \leftrightarrows AH^- + H^+$) is promoted by the pH' increase and by dilution. A similar interpretation has been given previously in the case pf other azocoupling products [9, 12b, 13].

The above given explanation shows however that the usual criterion to establish the aggregation nature of the equilibrium, namely the occurrence of isosbestic points by the dependence on concentration of the spectra, seems to be invalid in the case of the dyes **1**, **2**, **4**. Consequently, this criterion appears to be uncertain.

On the other hand, also the observed shift of the longest wavelength absorption maximum as a result of dye concentration decrease for all dyes (Figure 8c, 4b, 5b, 9) does not appear to be due to aggregation equilibrium, as main reason for dye concentration dependence. As shown in aggregation equilibrium of potentially tautomeric azocoupling products, the decrease of dye concentration should displace it towards the non-aggregated form [1b, 26, 28b]. Such a form, in the case of the dye **1**, *e.g.*, should correspond to the hydrazone **1b** that should lead to a shift towards 412.5 nm. In fact, we have noticed a reverse shift up to 442 nm (Figure 8c). Such a bathochromic shift (from 388 to 435 nm) is also observed with the dye **2**. If **2** would be involved in aggregation equilibrium, the band position shift should be a hypsochromic one, towards 388 nm. In reverse, the dyes **3** and **4** exhibit hypsochromic displacement of the bands as their concentration decrease (Figure 5b, 9). If the dyes **3** and **4** would be involved in aggregation, the shifts should be bathochromic. These displacements (**1**, **2**-bathochromic; **3**, **4**-hypsochromic) are rather compatible with the involvement of all dyes in acid-base equilibrium, corresponding to the first ionization step (Figure 8a, 4a, 5a, 6).

The influence of the temperature

The change of temperature within the range 20-60℃ influences slightly and differently the UV-Vis absorption spectra of the dyes **1**-**4** in ethanol solutions (*e.g.* Fig 8b, 10). In the case of dye **1** (Figure 8b), the absorption curves recorded at various temperatures exhibit isosbestic points and changes of the spectra by the temperature increase are reversible. This behaviour is characteristic to the presence of an equilibrium, which seems to correspond to acid ionization equilibrium **1b** ⇆ **7** since the absorption curves shown in Figure 8b are similar to some absorption curves recorded in pH' region of the first ionization step (Figure 8a).

On the other hand, the temperature dependence of the spectra of the dyes **2**-**4** do not exhibit isosbestic points and the change determined by the temperature increase is more or less regular and apparently not reversible (*e.g.* Figure 10). These experimental results show clearly that the temperature dependence of the spectra of the dyes **2**-**4** cannot be rationalized only by involvement of an acid-base equilibrium.

The influence of the time

The time dependence has been investigated by the recording of the UV-Vis absorption spectra of the dye **1** at various time intervals after preparation of the solution in absolute ethanol (Figure 11b, c). As a function of the ethanol used as solvent, purchased from two commercial sources, different sets of absorption curves have been obtained. The two sets differentiate by the position (Figure 11b, c; $\Delta\lambda_{max}$ = 22 nm) and shape of the visible absorption band that is characteristic for all the spectra belonging to a certain series of measurements. The spectra recorded with different sorts of ethanol differ among them as they were recorded at two different pH values (Figure 11a). The difference of the two series consist of the shift brought about by the different pH' values of the two sorts of ethanol [9b].

On the other hand, the set of absorption curves of dye **1** as a function of time in each experiment does not exhibit isosbestic points. A gradual increase of the absorbance by time since preparation of the solution takes place. The absorbance increase ceases within several hours and is apparently irreversible. These findings cannot be rationalized by the involvement of an acid-base equilibrium only. Other phenomena should cause this behaviour, namely some processes taking place more slowly than proton transfer [49]. The slow process in solution of an azocoupling product might be either aggregation or azo-hydrazone equilibrium, as asserted by nearly all the papers that describe the time dependence of the spectra of other azocoupling products [10, 20, 29, 30, 36].

Taking into account all the data presented in this work, the most reasonable cause for the time change of the UV-Vis absorption spectrum of the dye **1** in absolute ethanol is a slow disaggregating process $(\mathbf{1b})_n \rightarrow n\cdot\mathbf{1b}$. This explanation supposes that the absorption bands of the aggregate $(\mathbf{1b})_n$ and of hydrazone **1b** are superposed, and the absorption coefficient of the aggregate is lower than that of the hydrazone **1b**. Situation like that has been described previously in several aggregation studies of other azocoupling products [21, 23, 24]. In addition, the hydrazone **1b** released from the aggregate undergoes a rapid deprotonation **1b** ⇆ **7.** A deprotonation of aggregate itself is not excluded. Consequently, the aggregate, hydrazone **1b** and the anion **7** co-exist in ethanol solution of **1**. The proportion depends upon time and pH' of the sort of ethanol used. Due to the presence of the anion **7** (λ_{max} = 441.5 nm), the ethanol solution maximum is shifted bathochromically as compared to that of the aggregate and the hydrazone **1b** (λ_{max} = 412.5 nm). Such a disaggregating process can also justify the bathochromic shift by passing from a solution in absolute ethanol to that of ethanol- water mixture in the volumetric ratio 9:1.

Comparison of dye 1 spectrum in absolute ethanol and in solid state

Several authors consider that the dyes in solid state as crystals [50-52], as powder [1d, 23], adsorbed [1b, 20] or deposited [53] on a substrate, contain aggregates [1b, 1d, 20, 23, 50-53] along with the monomeric species.

This assessment is supported by the similar UV-Vis absorption spectra of a certain dye in solid state and in solution, when the aggregation in solution has been asserted [20, 23, 50, 5]. In fact, because the type of intermolecular interactions involved in the generation of the aggregates in solution [cf. 1b, 1e] and of the solid state are often the same for a certain dye, one expects that the spectra to be similar. In this context, the experimental findings that the UV-Vis absorption spectra of the dye **1** in absolute ethanol and in solid state are quite similar (Figure 12) might be considered [53] an argument for the existence of an aggregate $(\mathbf{1b})_n$ of dye **1** in absolute ethanol along with the forms **1b** and **7**.

CONCLUSIONS

The pH' influence on the UV-Vis absorption spectra of the azocoupling products **1**-**4** in aqueous ethanol is reliable explained by the involvement of each dye in two-steps acid ionization equilibria.

The comparative study of the pH' influence on the UV-Vis absorption spectra of the dyes **1**-**4** with the corresponding influence of other external factors on the spectra has revealed that: i) the water content, dye concentration or temperature dependence, with the occurrence of isosbestic points, may be adequately explain by the involvement of dye in the first-step acid ionization equilibrium; ii) the effect of the same factors and time on the spectra, when isosbestic points do not exist, is apparently due to superposition of the first-step acid ionization equilibrium and other phenomena.

The results of this work lead to the assessment that, when the effect of solvent, of dye concentration, temperature or time on the UV-Vis absorption spectra of potentially tautomeric azocoupling products is studied, the condition to ascertain as accurately as possible the nature of the involved equilibrium needs a comparison with the pH' influence.

EXPERIMENTAL SECTION

The synthesis and purification of the dyes **1**-**4** were performed as previously described [33,40-42,47]. The coupling component **5** and the nitrobenzenediazonium salts **6** were obtained according to our previously reports [40, 41, 47]. Analytical grade reagents and solvents were provided by Merck (Darmstadt), Fluka (Buchs), Chimopar and Cristal (Bucharest). These

were used without further purification. The water to prepare the solutions was distilled twice. As a rule, a relative concentrated stock solution (10^{-5}-10^{-4} mol·L^{-1}) of each dye **1**-**4** in ethanol was prepared. This solution was used as such or diluted with ethanol or water to the concentration requested by the UV-Vis spectrophotometric measurements (10^{-6}-10^{-5}mol·L^{-1}). The ionization constants (pK_a' values) were determined spectrophotometrically at 25^0 C, in the presence of 0.01 mol·L^{-1} KCl, on the basis of correlation between pH' and absorbance (figure 13) at analytical wavelength. [9, 16-19, 25]. The correlation was fitted with the aid of the programme *Table Curve Windows v. 1.10, Table Curve™, Jandel Scientific, Copyright 1989-1993 AISN Software*. The UV-Vis absorption spectra were performed on *Jasco-V-530 spectro-photometer*. Matched glass or quartz cells of 0.5, 1, 2 and 5 cm path lengths were used. The cells have been tightly sealed during the measurement when temperature or time dependence was followed. The experimental pH' values corresponding to the working conditions were measured by digital *Practronic MV-87 pH meter* equipped with a combined *pH-reference electrode ESHC-02*, produced by Naposenz SRL, Cluj-Napoca. The temperature was controlled by means of an ultra thermostat. The UV-Vis spectrum of **1** in solid state was recorded with a sample deposited as a solid film by application of drops of solution of **1** in chloroform on a quartz plate, followed by the complete evaporation of the solvent at room temperature. The IR spectra were recorded as KBr pellets on a *Jasco 615 ET-IR spectrometer.* The ^{1}HNMR spectra were performed with *FT-Bruker Avance 300 NMR spectrometer.*

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