Spectrophotometric pKa Values of 3-(*m*-Nitrophenyl)-1,5-Diphenyl Formazan (MNF) and 3-(*p*-Nitrophenyl)-1,5-Diphenyl Formazan (PNF) in Different MeCN-Water Binary Mixtures

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Summary: The acid dissociation constants of 3-(*m*-nitrophenyl)-1,5-diphenyl formazan (MNF) and 3-(*p*-nitrophenyl)-1,5-diphenyl formazan (PNF) have been determined in acetonitrile-water binary mixtures [50%, 60% and 70% (v/v)] by spectrophotometric method at 25 °C and an ionic strength of 0.1 mol $\cdot L^{-1}$ KCl. The absorption spectra were recorded in solutions of varying pH-values. Data evaluation was performed using the STAR software program which calculates stability constants and molar absorbances of the pure species by multilinear regression. A linear relationship between acidity constants and the mole fraction of acetonitrile in the solvent mixture was observed. The effect of solvent properties on acid-base behavior was discussed. The effect of nitro groups attached to para and meta positions on the ionization constant were investigated and the results were recorded.

Keywords: Formazan, acidity constants, acetonitrile-water mixtures, spectroscopic method.

Introduction

Formazans are compounds which contain the characteristic chain of atoms: N = N – C = N – NH and form a distinct class of organic compounds due to their importance in analytical chemistry [1, 2], biological and industrial applications [3-5]. Their structure was first reported by Bamberger [6] and by Von Pechmann [7], who agreed to call them “formazyl compounds”. Formazans substituted with specific acidic or basic groups show the behavior characteristic of these groups. 3-Nitro-1,5-diphenylformazan shows a strongly acid reaction, due to the ionization of the pseudo-nitro form [8].

The knowledge of pK_a values provides a basis for understanding the chemical reactions between the compound of interest and its pharmacological target [9, 10]. Additionally, they play a major role in acid-base titrations, complex formation and various analytical procedures. Also, the pK_a value(s) of a compound influences many characteristics such as its reactivity, spectral properties (color) and determination of the activity centers of enzymes in biochemistry [11].

The mostly used pK_a determination techniques are liquid chromatography [12, 13], potentiometry [14, 15] and ultraviolet-visible (UV-VIS) [16, 17] absorption**.** UV-Vis spectrophotometry is an attractive method for pK_a determination provided that the compound possesses pH-dependent light absorption [18]. Very often, the main difficulty in the determination of dissociation constants of compounds is their aqueous insolubility that forces the use of spectroscopic technique. This technique requires very low analyte concentration and allows suitable absorbance measurement in aqueous solution even for products with low aqueous solubility. Computer programs using data from multiwavelenght spectrophotometry are frequently used for the determination of acid dissociation constants [19, 20]. These programs often use principal component analysis and target factor analysis to analyze two and three-component kinetic systems, and the analyte concentrations are calculated by solving the simultaneous equations of mass balance.

The dissociation constants of ionizable analytes have been determined and discussed in terms of solvent characteristics by various authors in mixed solvents, such as methanol-water, acetonitrile (MeCN)-water, and tetrahydrofuran-water mixtures [21-25]. The influence of an organic solvent added to a medium on the dissociation of ionizable analytes is extensive in many cases and must be accounted for. The variation of the p*K* values with the content of the organic modifier can be explained by consideration of the preferential solvation of electrolytes in organic solvents. To elucidate the influence of a change in the medium on the systems studied and on retention in liquid chromatography, the values of the dissociation constants can be related to macroscopic parameters [cosolvent percentage, the mole fraction of cosolvent

(x), and the dielectric constant (ε)] and to microscopic parameters (Kamlet and Taft's solvatochromic parameters: α, solvent hydrogen-bond acidity; β, solvent hydrogen-bond basicity; π*, dipolarity/ polarizability) [26-28].

MeCN and its mixtures with water are widely used as a solvent, due to the excellent characteristics of the pure solvent. MeCN is a very weak base and a very weak acid and therefore it is a good differentiating solvent for both acids and bases. Furthermore, it has low viscosity and ideal good UV transparency. It has a relatively high dielectric constant (ε = 36) and a small autoprotolysis constant ($pK_s = 33.6$).

In the present report, pK_a values of two formazans; MNF and PNF (Fig. 1) were determined in 50%, 60% and 70% (v/v) MeCN-water mixtures at an ionic strength equal to 0.1 mol L^{-1} with KCl, to overcome the lack of information related to the acid-base equilibria of this kind of compound by means of spectrophotometric measurements.

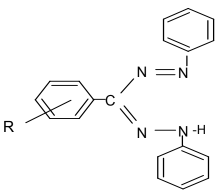

Fig. 1: The structure of formazans (R= *m*-NO_2, *p* -NO_2).

Results and Discussion

Formazans are compounds containing the characteristic azohydrazone group (HN = N – CH = N – NH_2), 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. 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 [29]. This may be attributed to the contribution of NH group in the formation of a hydrogen chelate ring with the participation of the azohydrazone tautomerism [30]. There have been several study concerning with the pKa values of substituted formazans. Grote *et al*. reported that the pK_a values of ortho-substituents formazans evaluated in mixtures of dioxane and water were usually higher than 11.00 [31]. Hutton and Irving found that the acidity constant of 3-Carboxymethylthio -1,5-diphenylformazan was 12.48 [32]. In a spectrophotometric study, Issa *et al* reported that the acid dissociation constants of some 1,5-diphenylformazan derivatives were in the range of 2.30 – 11.75 [33].

In this study, the pK_a values of the MNF and PNF in acetonitrile-water binary mixtures which determined by spectrophotometric method were listed Table-1 with standard deviation. Also UV-Vis spectra of MNF (%60 MeCN-water mixtures) and of PNF (%70 MeCN-water mixtures) at different pH values were given in Fig. 2 and Fig. 3, respectively. Its known that the acid-strengthening effect of the nitro group is greater in the para position due to the negative charge of the phenoxide ion is delocalized onto an oxygen of the nitro group and therefore PNF is more acidic than MNF.

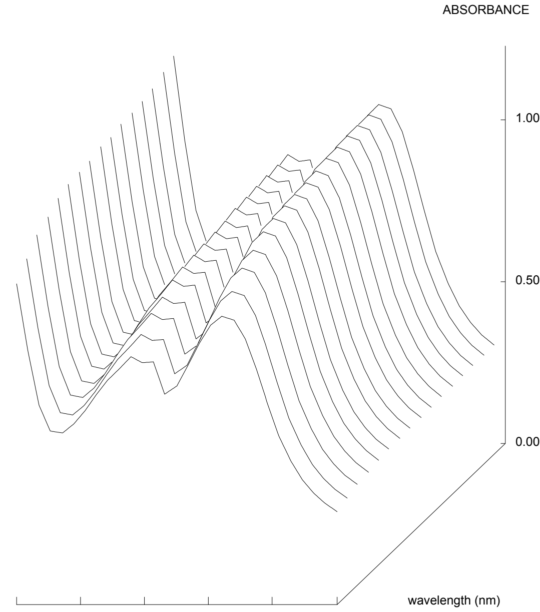

Fig. 2: The wavelength (nm)-absorbance graphic for MNF (in 60% MeCN-water binary mixtures).

Table-1: Dissociation constant of formazans.

Compound	50% MeCN-water	60% MeCN-water	70 %MeCN-water
PNF	10.146 ± 0.061	9.865 ± 0.130	9.533 ± 0.058
MNF	10.713 ± 0.061	10.618 ± 0.054	10.488 ± 0.043

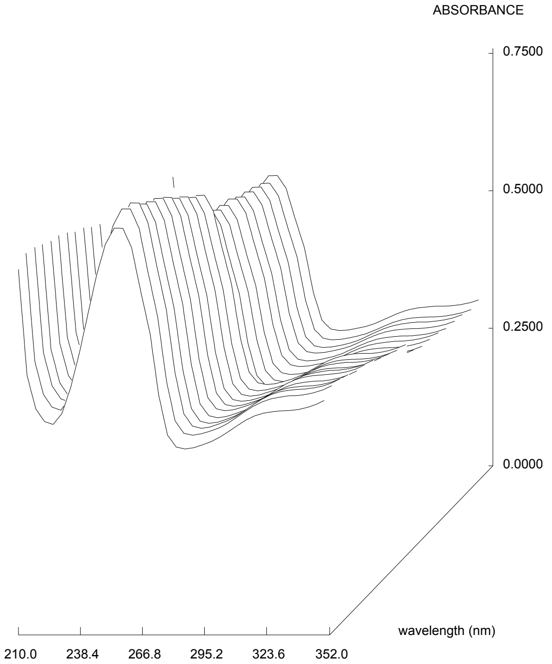

Fig. 3: The wavelength (nm)-absorbance graphic for PNF (in 70% MeCN-water binary mixtures).

It is known that one of the most important factors determining dissociation constants is the reaction medium and ionic strength. The variation of the pK_a values of studied compounds versus the mole fraction of MeCN, X_{MeCN}, in the MeCN-water mixtures is presented in Fig. 4. The equations between pK_a values and mole fraction of organic modifier are shown in Table-2. There is actually a linear relationship between the pK_a values of the studied compounds and X_{MeCN}, in the binary mixtures. The same trend was also observed for various organic molecules in different solvent mixtures by previous works [12, 18, 34]. It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of studied compounds on the solvent composition.

Table-2. The linear equation between experimental pK_a values and the mole fraction of the organic modifier.

Compounds	**Equation**	**Regression Coefficient**
PNF	**$y = 11.184\ (0.08) - 3.952\ (0.20)x^{a}$**	**R = 0.998**
MNF	**$y = -11.097\ (0.05) + 1.450\ x\ (0.14)$**	**R = 0.995**

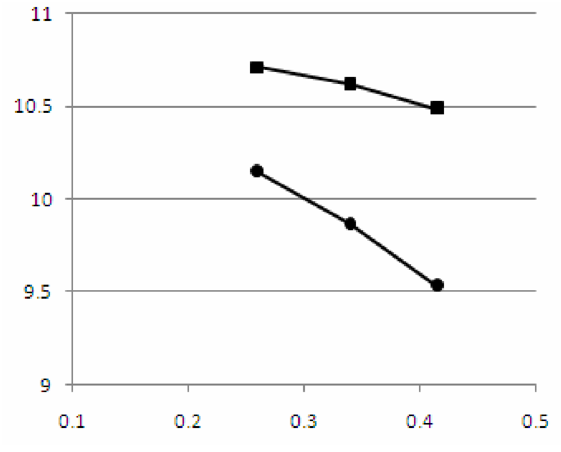

Fig. 4: pK_a values versus mole fraction of MeCN. (●) PNF and (■) MNF.

The influence of MeCN content on pK_a values of compounds was as expected: In all cases, there is a decrease in the pK_a values as the composition of MeCN increases in the acetonitrile-water binary mixtures. The pK_a values of compounds studied values showed changes in the range from 50% to ca. 70% MeCN. This is explained by the structural features of MeCN–water mixtures [26] and changes involved in the protolytic equilibria. The same trend has already been reported for various organic molecules in different solvent mixtures [35-37]. The different behavior of neutral bases can be explained on the basis of a theory [38] established by Izmailov *et al.*[39]. When the N group is deprotonated, there is no change in the number of charges involved in the process ($HA^{+} \leftrightarrow H^{+} + A$), because the change is from a positively charged species to a neutral one plus an hydrogen ion, and, therefore, a change in the polarity of the medium has a minor influence on the isoelectric dissociation reaction, which depends only on the solvation of the different species by the solvents of the mixture.

Experimental

Preparation of MNF and PNF

The formazan derivatives in this study were prepared by the coupling reaction of hydrazones (obtained by the condensation reaction of m-nitro aldehyde and p-nitro aldehyde with phenylhydrazine at pH 5.5) with benzendiazonium chloride in alkaline medium at 0–5 ^{o}C^{6}. The resulting red precipitate was purified by recrystallization from ethanol until a sharp melting point (180°C for MNF, 196°C for PNF) was reached and no impurities on TLC plates were observed. The structures of the purified compounds were elucidated by using IR, ^{1}H-NMR and elemental analysis. Dimethylsulfoxide (DMSO) used was an absolute dry (water ≤ 0.01 %) batch of Fluka (41648) kept on beads of a molecular sieve (4A°).

Chemical and Reagents

The studied compounds (Fig. 1) were synthesized and the procedures of synthesis were described above. Analytical reagent grade chemicals were used, unless otherwise indicated. MeCN (Sigma, HPLC grade) were used without further purification. Potassium hydroxide (Titrisol), sodium hydroxide, hydrochloride acid (Titrisol), potassium hydrogen phthalate (dried at 110 ◦C before use), and potassium chloride (ionic strength adjuster; 0.1 mol L^-1) were supplied from Merck. All stock solutions of hydrochloride acid, potassium chloride and potassium hydrogen phthalate were prepared by water. Water, with conductivity lower than 0.05 μScm^{-1} was obtained with a Zeneer Power I (Human Corp.).

Apparatus

Potentiometric measurements were performed with Mettler-Toledo MA 235 pH/ion (resolution ±0.1 mV) analyzer system. All titrations were carried out under N_2 and at 25.0±0.1°C, which was maintained by circulating water from a constant-temperature thermostat (Heto CBN 8-30 and temperature control unit Heto HMT 200) through the double-wall *Pyrex* titration cell of 80-mL capacity. The UV-Vis absorbance spectra were recorded at each pH using Perkin Elmer LAMBDA 25 spectrophotometer, equipped with 1.0 cm path length quartz cell with a fixed slit width (2 nm), controlled by personal computer. A peristaltic pump equipped with the spectrometer was used to circulate the solution from the titration vessel to the spectrophotometer cell, and vice versa, through Teflon or Tygon tubes in a closed loop circuit with continuous flow.

Procedure

Before the spectrophotometric titration, carbonate-free potassium hydroxide solutions were prepared under a nitrogen atmosphere. The ionic strength of KOH solution was adjusted to 0.10 mol L^{-1} by the addition of KCl. The alkali titre and absence of carbonate were periodically checked by pH-metry, using the appropriate Gran function [40, 41] against primary standard oven-dried potassium hydrogen phthalate. The pK_a values of the MNF and PNF were determined by means of the data obtained from spectrometric titrations in 50 %, 60 % and 70 % (v/v) MeCN–water mixtures at 25.0±0.1°C and in 0.1 mol L^{-1} ionic strength (KCl). The spectrophotometric multiple-wavelength pH-titration was carried out as follows: in a first step, the standard emf values, E^o, of the potentiometric cell were evaluated from titrations of a measured amount of an acidic solution, at the same conditions of temperature, ionic strength and solvent composition to be used in later experiments using KOH solutions in the same solvent and ionic strength as the titrant, and checking the calibration parameters from the Gran plots [40, 41]. The standard emf of the cell, E^o, is the average of at least 5 standardizations. The standardization of the electrode system was carried out, each time solvent media was changed and the constancy of E^o, values ensured by continual surveillance by means of periodic calibrations.

In a second step, a solution of fully protonated compounds (30.0 mL containing 1.10^{-5} $mol.L^{-1}$ compounds) by HCl at the required conditions of temperature, ionic strength and solvent composition were titrated using KOH solutions in the same solvent and ionic strength in the pH range of 3.0-12.0. After each addition, the potential was allowed to stabilize, its value used, in combination with E^o calculated in calibration step, to calculate the pH solution.

In the UV-Vis spectrometric titrations, the test solution was pumped to a spectrometric flow-cell by means of a peristaltic pump. After each addition of titrant, and after waiting for the emf reading to be stable, a spectrum, UV-Vis spectra were recorded with 1 nm resolution at 210-350 nm intervals in order to obtain different spectra around the maximum λ for each com.

Data Treatment

Spectrometric titrations data were processed using the program STAR (Stability Constants by Absorbance Readings) which calculates stability constant and molar absorbtivities of the pure species by multilinear regression [42]. The program STAR requires a previous model of the chemical equilibria, based upon the existence of certain chemical species, to be postulated. The refinement of equilibrium constants is done using the Gauss-Newton non-linear least squares algorithm by numerical differentiation, until a minimum in the sum of the squares residual (U) is attained. Wavelength (U_{abs}) is obtained:

$$U_{abs} = \sum_{i=1}^{ns} \sum_{j=1}^{nw} (A_{i,j,\exp.} - A_{i,j,calc.})^2 \qquad (1)$$

Where ns and nw indicate the number of spectra and wavelengths, respectively. $A_{i,j,exp}$ and $A_{i,j,calc}$ are the experimental and calculated absorbance values for the wavelength j in the spectrum i. The calculated absorbances are obtained in three steps: the program first solves the mass balances for each spectrum according to the guessed equilibrium constants and experimental conditions; then, a multiple linear regression procedure is applied in order to determine the molar absorbances of each unknown species, and finally the individual absorbance values are re-calculated from the guessed species concentration and the corresponding molar absorbances. The optimization is performed by means of a non-linear least-squares procedure. The minimization process is repeated until the relative change of U between two iterations is ≤0.01% [43].

Conclusions

In this study, the pK_a values of MNF and PNF have been determined precisely with spectrophotometric titrations in various MeCN–water binary mixtures. This work presents the first study dealing with the determination of pK_a values of these compounds in MeCN-water mixtures

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