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Some 2-(2-Thiazolylazo)-4-methoxyphenol (TAMP) Complex Equilibria. I. Acid-base Properties of TAMP in Water and in Various Mixed Solvents

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The dissociation constant of 2-(2-thiazolylazo)-4-methoxyphenol (TAMP) in 0.1 M KNO_3 at 25.0°C in aqueous solution, as well as in various mixed solvents was determined by means of spectrophotometry. The data were treated by graphical methods as well as by a computer using the general minimizing program LETAGROP-SPEFO. A simple program for evaluation of dissociation constants from spectrophotometric data has also been developed and compared with LETAGROP. The protonation constant of TAMP in water and 30 % ethanol has also been determined.

TAMP and some other dyes of the same group were prepared by Kawase [1] in 1962. He also studied its complexes with some divalent ions [2,3] as well as Co(III).[4] Later Chromý and Vřeštál [5] used TAMP for complexometric determination of Sn(II) in organic compounds. Chromý and Sommer [6] studied TAMP as a complexometric indicator for the determination of some di- and tri-valent metal ions and they worked out several methods for their determination. Sommer, Šepel, and Ivanov [7] showed that TAMP was a very sensitive spectrophotometric reagent for the determination of uranyl ion and deduced composition and some other properties of the complex UO_2R^+ (R = TAMP). Recently Kai [8] used TAMP for the spectrophotometric and complexometric determination of mercury(II) in solution and found two complexes with molar ratios M : R equal to 1 : 1 and 1 : 2. More recently, Chromý and Sommer [9,10] studied complexes of Cu(II) with TAMP, and some of its derivatives in aqueous solution.

* The experimental work and the graphical treatment of the data were carried out by V. K. in Brno, the computer adjustment by J. H. in Stockholm. Permanent address: the same as for V. K.

In order to evaluate the complex formation between TAMP and various metal ions it is necessary to know its acid-base properties in the solvents studied. For that reason we have determined the dissociation constant of TAMP in water as well as several mixed solvents with water as one component.

The solvents studied were: H_2O, 30 % CH_3OH, 30 % DMF, 10, 20, 40, and 50 % C_2H_5OH. The experimental data have been treated with several calculation methods and the results of the calculations compared.

In order to check that the protonation equilibrium of TAMP does not interfere with the complex formation of metal ions down to pH≈1, *e.g.* the complex equilibria with Ni(II),[47] some experiments have been performed at high acidities in H_2O and 30 % C_2H_5OH. The data for the protonation of TAMP and the data for the evaluation of the dissociation constant of TAMP in 20 % and 40 % ethanol were treated by graphical methods only.

EXPERIMENTAL

Reagents and solutions. TAMP was prepared by Svoboda and Bendová [29] (Institute of Pure Chemicals, Lachema, Brno) in a form of bright, darkgreen crystals, sparingly soluble in water but very soluble in dimethylformamide (DMF) and alcohols, giving yellow-orange solutions. It was recrystallized twice from hot ethanol, dried and 99.31 % of the dye was found by elemental analysis of C, H, N in this preparation.

The purity of TAMP was further checked by thin layer chromatography on silica gel (MN-Kieselgel G) using the following mixtures as eluents: 1, 20 ml benzene + 5 ml $CHCl_3$ + 9 ml diethylether + 0.3 ml conc. acetic acid; 2, 33 ml CCl_4 + 18 ml diethylether + 3 ml conc. acetic acid; 3, 35 ml benzene + 4 ml $CHCl_3$ + 4 ml conc. acetic acid. In all cases one spot was found;[30] *i.e.* the dye was chromatographically pure.

A stock solution of TAMP ($c_R = 5 \times 10^{-4} - 1 \times 10^{-3}$ M) was prepared by dissolving the dye in 2.5 ml DMF and 2.5 ml 1 M NaOH and dilution with water up to the mark so that the DMF concentration was not larger than 1 % (*v/v*) in the stock solution and 0.1 (or 0.2 %) (*v/v*) in the others. Traces of heavy metal ions were masked by adding a solution of EDTA.

pH Control. The pH in the solutions used was adjusted by mixing in a nitrogen atmosphere the acid solution of the reagent (pH≈1.1) with an alkaline solution (pH = 11 – 12) containing the same concentration of TAMP. The equipment used permitted continuous measurements of spectrophotometric curves.[31,32] More details about this technique can be found elsewhere.[33] The pH meter (Radiometer, Type S-1001) was calibrated by phosphate (pH = 6.48 at 25°C), phthalate (NBI, pH = 4.01 at 25°C) and tetraborate (NBI, pH = 9.18 at 25°C) buffer solutions. The pH values in mixed solvents were not corrected and we use the symbol pH in all cases.

Ionic strength. Constant ionic strength $I = 0.10$ was maintained during these studies by addition of potassium nitrate (twice recrystallized) or prepared from a mixture of nitric acid and sodium hydroxide.

All other chemicals and solvents were commercial products of *p.a.* purity and purified by recrystallization, distillation *etc.*, before use.

Instruments. All spectrophotometric measurements were made at constant temperature (25.00 ± 0.05°C) on the one beam spectrophotometer SFD-2. Absorption spectra were registered on a UNICAM SP 700 recording spectrophotometer at room temperature. The pH values of the solutions used were measured with a Radiometer PHM-4d pH meter with a precision of ± 0.02 pH unit using glass G 202B and saturated calomel K 401 electrodes (Radiometer).

RESULTS

Table 1a gives a survey of the titrations in the solvents used. Table 1b gives the experimental data for one typical titration in water together with the

Table 1a. Survey of experiments. $t = 25°C$, $I = 0.1$ M (KNO_3).

Set No.	Solvent	C_R M	C_{EDTA} M	pH-range
1	H_2O	5.00×10^{-5}	0	6.26 – 10.30
2	H_2O	5.00×10^{-5}	2×10^{-4}	6.05 – 11.63
3	H_2O	1.00×10^{-4}	4×10^{-4}	6.20 – 9.98
4	H_2O	2.00×10^{-4}	6×10^{-4}	6.09 – 10.04
5	30 % DMF	5.00×10^{-5}	2×10^{-4}	6.20 – 10.34
6	30 % CH_3OH	5.00×10^{-5}	2×10^{-4}	6.20 – 10.15
7	10 % C_2H_5OH	5.00×10^{-5}	2×10^{-4}	6.29 – 9.87
8	50 % C_2H_5OH	5.00×10^{-5}	2×10^{-4}	7.08 – 11.03
9[a]	H_2O	4.50×10^{-5}	0	0 – 1.8
10[a]	H_2O	4.50×10^{-5}	0	0.15 – 1.51
11[a]	30 % C_2H_5OH	4.50×10^{-5}	0	0.03 – 1.92
12[a]	30 % C_2H_5OH	4.50×10^{-5}	0	0.14 – 1.66
13	20 % C_2H_5OH	5.00×10^{-5}	2.00×10^{-4}	5.12 – 11.50
14	40 % C_2H_5OH	5.00×10^{-5}	2.00×10^{-4}	5.20 – 12.00

[a] The ionic strength not constant but varied around 0.1 M.

Table 1b. Experimental data (pH; A_{exp}) for the dissociation of TAMP in water. For all points deviations $\Delta A = 10^3(A_{calc} - A_{exp})$ are given, using the dissociation constants and molar absorptivities determined by LETAGROP-SPEFO (see Tables 2 and 3). $I = 0.10$ M KNO_3; $t = 25°C$. Set No. 1.

pH	A $\lambda=500$	ΔA	A $\lambda=525$	ΔA	A $\lambda=540$	ΔA	A $\lambda=560$	ΔA	A $\lambda=580$	ΔA	A $\lambda=610$	ΔA
6.26	0.276	−4	0.153	−1	0.097	+1	0.057	−2	0.040	+5	0.027	+7
6.40	0.277	−4	0.160	−3	0.103	+1	0.070	−3	0.048	+3	0.033	+5
6.53	0.278	−4	0.167	−4	0.113	−2	0.075	+2	0.056	+3	0.040	+3
6.63	0.278	−4	0.170	−2	0.121	−2	0.085	0	0.066	+1	0.047	+1
6.73	0.279	−3	0.177	−2	0.131	−3	0.096	−1	0.078	−1	0.055	−1
6.87	0.279	−1	0.190	−3	0.147	−3	0.115	+2	0.095	−2	0.065	0
6.96	0.285	−1	0.200	−3	0.160	−3	0.132	−1	0.111	−4	0.075	−1
7.03	0.286	0	0.207	−2	0.170	−2	0.145	+1	0.121	−2	0.084	−3
7.14	0.290	+1	0.222	−1	0.193	−4	0.169	0	0.147	−7	0.099	−4
7.23	0.295	0	0.237	+1	0.210	−2	0.191	0	0.165	−4	0.113	−5
7.35	0.297	4	0.257	+1	0.240	−2	0.224	+1	0.195	−3	0.131	−3
7.42	0.301	4	0.272	1	0.258	−2	0.245	0	0.218	−6	0.146	−5
7.51	0.305	6	0.292	1	0.285	0	0.277	3	0.246	−6	0.163	−4
7.60	0.315	2	0.315	−1	0.313	−2	0.305	2	0.274	−4	0.180	−2
7.70	0.318	6	0.331	8	0.347	5	0.340	4	0.306	−1	0.203	−3
7.79	0.323	7	0.358	4	0.377	5	0.372	1	0.337	1	0.221	−1
7.90	0.330	8	0.382	9	0.415	7	0.415	−2	0.372	6	0.245	0
8.01	0.340	6	0.412	6	0.451	5	0.459	0	0.409	5	0.268	2
8.10	0.349	2	0.430	9	0.480	10	0.488	6	0.440	5	0.290	−2
8.23	0.358	1	0.462	5	0.517	7	0.531	−3	0.480	4	0.314	−1
8.35	0.362	4	0.483	7	0.541	6	0.571	−2	0.512	3	0.336	−3
8.44	0.365	5	0.501	4	0.560	7	0.590	−1	0.529	7	0.345	1
8.51	0.370	3	0.510	5	0.578	3	0.610	−1	0.549	1	0.357	−1
8.63	0.376	1	0.529	1	0.600	1	0.630	0	0.570	2	0.370	−1
8.73	0.380	0	0.540	1	0.610	5	0.651	−1	0.581	5	0.378	0
8.82	0.385	−3	0.550	−1	0.629	−4	0.663	−2	0.598	−1	0.385	0
8.94	0.387	−3	0.558	−1	0.635	2	0.672	0	0.601	−1	0.394	−1
9.12	0.390	−3	0.570	−3	0.652	−2	0.690	3	0.621	2	0.398	4
9.23	0.391	−3	0.575	−3	0.658	−2	0.702	4	0.630	−1	0.404	2
9.38	0.395	−5	0.585	−8	0.672	−9	0.710	7	0.640	−4	0.408	2
9.53	0.396	−4	0.590	−9	0.675	−8	0.712	−1	0.650	−9	0.410	2
9.81	0.400	−5	0.597	−9	0.688	−12	0.725	−4	0.653	−5	0.413	3
10.30	0.422	1	0.603	3	0.692	34	0.738	−2	0.660	21	0.419	0

difference in absorbance $\Delta A = 10^3\ (A_{calc} - A_{exp})$ obtained by LETAGROP using the dissociation constants and molar absorptivities given in Tables 2 and 3.

The full experimental material covering the dissociation of TAMP in the solvents water, 30 % DMF, 30 % methanol, 10, 20, 40, and 50 % ethanol as well as the protonation of TAMP in water and 30 % ethanol can be obtained upon request.

Treatment of data

Within the last decades many numerical and graphical methods have been employed for the determination of equilibrium constants from spectrophotometric data.[11–17] Although the graphical methods are easy to use and give good results as long as the number of parameters to be determined does not exceed three they have the disadvantage of being laborious and time consuming. This can be remedied by letting a computer calculate the normalized functions to be used.[41]

It is difficult, however, to get good estimates for the standard deviations of equilibrium constants and molar extinction coefficients of the complexes under study.

Several computer programs have been developed for the evaluation of spectrophotometric data,[20–24] employing least-squares methods. All these methods suffer from being developed for surprisingly simple special cases such as the formation of a complex AB from A and B.[20] An attempt of a method generally applicable to the formation of mononuclear as well as polynuclear species, even with several ligands, is the program LETAGROP [35,45,46] developed by Sillén and coworkers and applied to many different kinds of equilibria.[42–44] Recently a version called LETAGROP-SPEFO was developed for the treatment of spectrophotometric data.[25,26] A good survey can be found in the literature.[18,19,28,36,37]

In the following the results from graphical as well as the computer programs LETAGROP-SPEFO and PRCEK, the latter one developed by the present writers,[34] are given.

I. Graphical treatment of data. The principles of the graphical methods we have been using for some time may be found elsewhere.[15–17] For the sake of brevity we shall give only the final equations. The transformations are derived from the equation for the equilibrium constant and mass balance equations, combined with the equation for total absorbancy of the solution (validity of Lambert-Beer's law is assumed, of course). After simple rearrangement and elimination of unknowns one obtains for the equilibrium (A) the following transformations:

$$\underset{\varepsilon_1}{RH_x} \rightleftharpoons \underset{\varepsilon_2}{RH_{x-q}} + qH^+ \qquad \text{(A)}$$

$$A = \varepsilon_1 c_R + (\varepsilon_2 c_R - A)[H^+]^{-q} K_x = A_{01} + F_1 K_x \qquad (1)$$

$$A = \varepsilon_2 c_R - (A - \varepsilon_1 c_R) K_x^{-1} [H^+]^q = A_{02} - F_2 K_x^{-1} \qquad (2)$$

$$c_R/A = 1/\varepsilon_1 - (c_R \varepsilon_2 - A)[H^+]^{-q} A^{-1} \varepsilon_1^{-1} K_x = \varepsilon_1^{-1} - Q_1 [H^+]^{-q} \qquad (3)$$

$$c_R/A = 1/\varepsilon_2 + (A - c_R\varepsilon_1)[H^+]^q A^{-1}\varepsilon_2^{-1}K_x^{-1} = \varepsilon_2^{-1} + Q_2[H^+]^q \quad (4)$$

where A is absorbancy, c_R the total reagent concentration, ε_1 and ε_2 are molar absorption coefficients, K_x is the equilibrium constant of the reaction (A), and the other symbols are self-explanatory. K_1 is the dissociation constant of TAMP and K_2 the dissociation constant of its protonated form.

By plotting $A = f(F_1$ or $F_2)$ or $c_R/A = f(Q_1[H^+]^{-q}$ or $Q_2[H^+]^q)$ straight lines are obtained for the right q value. The number of protons liberated in the reaction (A) may thus be evaluated by inserting various values for q. The values for the molar absorptivities and the equilibrium constant can then be obtained from the intercept (ε_1 and ε_2) and from the slope (K). More precise values of K_x and q could be evaluated by using "the graphical logarithmic analysis". The corresponding equation can be derived from any of eqns. (1) – (4) in the form (5)

$$\log \frac{A - \varepsilon_1 c_R}{\varepsilon_2 c_R - A} = \log K_x + q\mathrm{pH} \quad (5)$$

The slope of the plot $\log [(A - A_{01})/(A_{02} - A)] = f(\mathrm{pH})$ gives the value of q, and the value of pH for which $\log [(A - A_{01})/(A_{02} - A)]$ is equal to zero determines the value of pK/q, where $pK = -\log K_x$.

An example of graphical analysis of this type may be found in Figs. 1 and 2. From Figs. 1a and 1b for the protonation of TAMP in H_2O it can be seen, when applying equation (3) (Fig. 1a) that a linear relationship is obtained for $q = 1$. This value is then proved by applying equation (5) as can be seen from the unique slope of the line in Fig. 1b. Similarly, the dissociation constant of TAMP can be found graphically as illustrated in Fig. 2.

The procedure described and illustrated above, was applied separately to each wavelength and from the series of pK_x values mean values were calculated, which are listed in Tables 2 and 6.

Deviations from the mean $p\bar{K}$ values were evaluated using eqn. (6) [40]

$$\sigma(pK) = \left[\frac{1}{N_\lambda}\sum_{n=1}^{N_\lambda}(p\bar{K} - pK_n)^2\right] \quad (6)$$

where $p\bar{K}$ is the mean value calculated from pK values obtained from curves for individual wavelength n, N_λ is the number of wavelengths used. For better comparison with standard deviations computed by PRCEK (*cf.* below) and LETAGROP-SPEFO, the values $3\sigma(pK)$ have been listed, as well.

II. Treatment of the data by linear least-squares method (program PRCEK). In just the same way, as by means of the graphical method discussed in this paper, the "best" lines of transformations (1) and (2) could be found objectively using the linear least-squares method with help of a digital computer. Applying proper formulae one can evaluate standard deviations for molar absorptivities as well as for equilibrium constants. The values of ε_1 or ε_2 show a slight interdependence. Thus, one has to use repeatedly eqns. (1) and (2), until the values of the intercept $\varepsilon_2 c_R$ or $\varepsilon_1 c_R$ and the slopes are not altered significantly or, better, the changes are within certain limits. We did stop the calculations, if two consecutive values differed less than 0.1 rel. percent. By the use of a computer this can be achieved very easily. For the final lines obtained, the standard

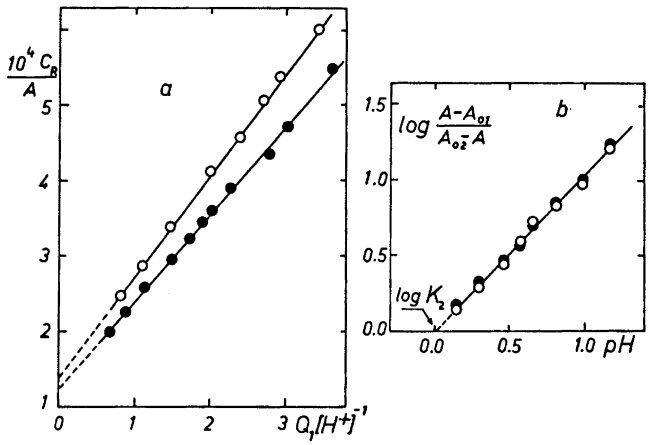

Fig. 1. Graphical analysis of the protonation of TAMP in H_2O. *a.* $10^4\ c_R/A$ plotted against $Q_1[H^+]^{-1}$ for two different wavelengths and $c_R = 4.50 \times 10^{-5}$ M. According to eqn. (3) straight lines correspond to $q = 1$ and the value ε_2 used (ε of RH form in this case) was equal to 800 and 1800. Open symbols correspond to 560 nm, filled symbols to 540 nm. *b.* log $[(A-A_{01})/(A_{02}-A)]$ plotted against pH for the same wavelengths as in Fig. 1a. According to eqn. (5) the extrapolation to $(A-A_{01})/(A_{02}-A) = 0$ gives $-\log K_2$, which should be independent of wavelength. The difference at the two wavelengths used indicates a slight systematic error, smaller than the experimental uncertainty.

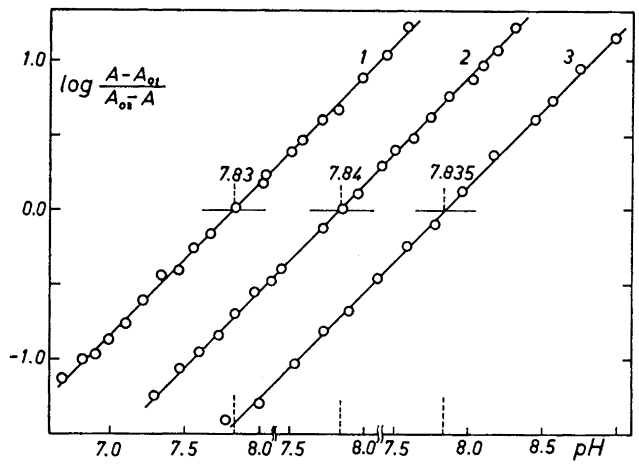

Fig. 2. Graphical determination of the dissociation constant of TAMP in water employing eqn. (5) for wavelength 560 nm. The values for ε_1 and ε_2 were obtained by graphical extrapolation using eqns. (3) and (4).

Curve 1: $c_R = 5.00 \times 10^{-5}$ M, $\varepsilon_1 = 1000$, $\varepsilon_2 = 14\ 520$. Curve 2: $c_R = 1.00 \times 10^{-4}$ M, $\varepsilon_1 = 810$, $\varepsilon_2 = 15\ 350$. Curve 3: $c_R = 2.00 \times 10^{-4}$ M, $\varepsilon_1 = 810$, $\varepsilon_2 = 15\ 350$. The lines have been plotted separately for the sake of clarity.

deviations of molar absorptivities and equilibrium constants were always calculated.

We have written a small Algol program called PRCEK [34] (DWARF in English) to achieve this. It contains some safeguards to avoid possible erratic behaviour. In the program one has to find some starting values for molar absorptivity coefficients, ε_1 in eqn. (1) and ε_2 in eqn. (2). We have taken the values found in a previous graphical analysis but they could be inferred from the graphs $A = f(pH)$, as well.

It is shown elsewhere,[34] that even starting with a quite poor estimate (several times higher or lower) the proper ε values are quickly found after a few successive cycles. The experimental points near the absorbancy value $A_{01} = \varepsilon_1 c_R$ or $A_{02} = \varepsilon_2 c_R$ usually cause trouble due to the fact, that even small experimental errors in absorbancy cause a large scatter of the functions F_1 or F_2. Therefore in the program the points for which the difference $|A - \varepsilon_1 c_R|$ or $|A - \varepsilon_2 c_R|$ was less than 10 (sometimes we have used even 20) % of the $|\varepsilon_1 - \varepsilon_2|\ c_R$ value, were discarded and the "best" lines were calculated from the rest of the experimental data.

The process was repeated for each wavelength and for the final values of K, and $\sigma(K)$, the average numbers were taken. Finally, the error-square sum

$$U = \sum_{N_p} \sum_{N_\lambda} (A_{calc} - A_{exp})^2 \qquad (7)$$

was calculated. N_p is the number of points at each wavelength, N_λ is the number of wavelengths and A_{calc} is the value of absorbancy, calculated from eqn. (8)

$$A_{calc} = (\varepsilon_1 [H^+]^q + \varepsilon_2 K) c_R ([H^+]^q + K)^{-1} \qquad (8)$$

Tables 2 and 3 give some values obtained in this way.

III. Treatment of the data by the program LETAGROP-SPEFO. The values of K found graphically for different media were used as starting values for the LETAGROP refinement.

The program searches for the "best" set of unknown parameters (equilibrium constants) K_1, K_2,...K_N (in our case $N = 1$ or 2 only) and another series of parameters ε_1, ε_2,...ε_N-molar absorptivities of individual species for individual wavelengths. Such a "best" set is defined as the one which gives the minimum value to the error square sum as defined above in eqn. (7).

One can choose by using a value of *val* (1 or 2) the error square sum to be minimized either on $U = \sum (A_{calc} - A_{exp})^2$ (absolute errors, $val = 1$), or $U = \sum [(A_{calc} - A_{exp})/A_{exp}]^2$ (relative errors, $val = 2$). We have used $val = 1$ in all calculations; in this way the experimental points have been given the same weight as with PRCEK, *i.e.* all points have the same weight.

In Table 2 the resulting values of $\log K \pm 3\sigma(\log K)$ are given, where $\sigma(\log K)$ is defined by

$$\sigma(\log K) = \tfrac{1}{2} \log \frac{K + \sigma(K)}{K - \sigma(K)}.$$

Table 2. Dissociation constants of TAMP determined by graphical method, PRCEK and LETAGROP-SPEFO programs. ($I = 0.10$ M KNO_3; $t = 25$°C. The experimental conditions for each set are given in Table 1a).

Set No.	Medium	$-\log K_1$[a] Graph.	$-\log K_1$	$U \times 10^3$ PRCEK	$-\log K_1$	$U \times 10^3$	$\sigma(A) \times 10^3$ LETAGROP	$\Delta \log K_1$[b]
1	H_2O	7.83 ± 0.03	7.830 ± 0.010	9.954	7.815 ± 0.007	3.240	± 4.12	+ 0.015
2	H_2O	7.83 ± 0.03	7.827 ± 0.015	5.395	7.836 ± 0.009	2.880	3.91	− 0.009
3	H_2O	7.84 ± 0.03	7.855 ± 0.013	5.140	7.861 ± 0.010	2.090	4.11	− 0.006
4	H_2O	7.83 ± 0.03	7.859 ± 0.019	13.838	7.858 ± 0.012	7.019	9.14	+ 0.001
Mean value		7.83 ± 0.03	7.846 ± 0.016	–	7.852 ± 0.010	–	–	–
All data together		–	–	–	7.858 ± 0.009	2.619	7.93	–
5	30 % DMF	8.38 ± 0.02	8.382 ± 0.014	10.564	8.386 ± 0.006	2.376	± 3.26	− 0.004
6	30 % CH_3OH	8.16 ± 0.03	8.156 ± 0.011	6.435	8.159 ± 0.006	2.107	3.07	− 0.003
7	10 % C_2H_5OH	7.92 ± 0.04	7.923 ± 0.012	3.298	7.922 ± 0.009	2.467	4.46	+ 0.001
8	50 % C_2H_5OH	8.92 ± 0.03	8.921 ± 0.022	11.389	8.925 ± 0.016	8.576	8.49	− 0.004

[a] Values resulting as a mean from the values calculated for each wavelength using eqn. (5).
[b] $\Delta \log K_1 = -\log K_1$ (PRCEK) + $\log K_1$ (LETAGROP).

Table 3. Molar absorptivities of R^- and RH forms of TAMP determined from the horizontal parts of $A = f(pH)$ curves, by graphical method, and by PRCEK and LETAGROP-SPEFO programs.

Set No.	λ (nm)	$\varepsilon_i = A_{01} i / c_R$[a] $\varepsilon_2(R^-)$	$\varepsilon_1(RH)$	Graphical method[a] ε_2	ε_1	PRCEK $\varepsilon_2 \pm \sigma(\varepsilon_2)$		$\varepsilon_1 \pm \sigma(\varepsilon_1)$		LETAGROP-SPEFO $\varepsilon_2 \pm \sigma(\varepsilon_2)$		$\varepsilon_1 \pm \sigma(\varepsilon_1)$	
1	500	8 010	5 400	7 950	5 450	7 980	± 20	5 485	± 19	7 890	± 21	5 370	± 24
	525	12 040	3 060	11 950	2 950	11 850	24	2 670	23	11 830	27	2 840	28
	540	13 800	1 740	13 700	1 720	13 650	36	1 855	43	13 630	29	1 680	30
	560	14 600	1 140	14 400	940	14 020	35	920	33	14 510	28	900	29
	580	13 100	800	13 150	710	13 030	32	700	44	13 110	30	620	31
	610	8 300	540	8 330	440	8 250	26	350	38	8 430	26	510	27
2	440	2 680	7 000	2 700	7 120	2 690	21	7 230	43	2 660	15	7 190	16
	470	4 120	7 460	4 200	7 550	4 190	11	7 560	21	4 160	14	7 500	14
	510	9 780	4 600	9 720	4 650	9 650	25	4 640	27	9 600	26	4 560	26
	540	14 000	1 880	13 870	1 770	13 720	44	1 830	45	13 740	31	1 780	32
	560	14 880	1 040	14 520	1 000	14 640	58	910	25	14 690	32	930	32
	580	12 800	700	12 700	620	13 160	50	620	41	13 230	31	640	31
	610	8 460	360	8 350	390	8 410	21	450	28	8 530	31	570	31

[a] Standard deviations have not been estimated for molar absorptivities found from horizontal parts of $A = f(pH)$ curves and by graphical methods.

Moreover, $\sigma(A)$ the standard deviation in A is given. Molar absorptivities ε_i together with their standard deviations $\pm\sigma(\varepsilon_i)$ are given in Table 3 for one typical run in water.

The possible existence of the complex $R(RH)^-$. By LETAGROP one can very easily try to introduce a new species and see whether this addition can significantly improve the fit or not. It is made possible in a quite elegant way [35] by a "species selector" in the program.

Table 4. Values of error square sum U_{min}, logarithms of constants K_1 and K_{12} (assuming species R^- and $R(RH)^-$, respectively).

Set No.	10^3U	$\sigma(A)\times 10^3$	pK_1	$10^3\times U$	$\sigma(A)\times 10^3$	pK_1	pK_{12}
	assuming only reaction (A)			assuming reaction (A) and (B)			
1	3.240	±4.12	7.815±0.007	0.930	±2.25	7.98(max 7.76)	4.28±0.20
2	2.880	3.91	7.836±0.009	2.258	3.54	7.88±0.06	4.72±0.21
3	2.090	4.11	7.861±0.010	–	–	7.78±0.20	rejected
4	7.019	9.14	7.858±0.012	–	–	7.88±0.06	rejected
	26.193[a]	7.93	7.858±0.009	–	–	7.87±0.02	rejected
5	2.376	3.26	8.386±0.006	2.336	3.30	8.39±0.01	6.18±0.17
6	2.107	3.07	8.159±0.006	–	–	8.19±0.03	rejected
7	2.467	4.46	7.922±0.009	2.098	4.22	7.92±0.02	4.89(max 4.67)
8	8.576	8.49	8.925±0.016	–	–	–	K < 0; rejected

[a] All data together.

The new species added is accepted, if U_{min} is significantly lower than the previous one and if the constant for the new complex is coming out with a standard deviation $\sigma(K)$, for which the condition $K > F_\sigma \times \sigma(K)$ is fulfilled. F_σ is the value for *Sigfak* in the program. We have used $F_\sigma = 1.5$, *i.e.* the confidence level 93 %.

It was of some interest to test the formation of a species $R(RH)^-$. In series 1 and 2, *i.e.* for $c_R = 5\times 10^{-5}$ M the species was accepted (see Table 4), U_{min} decreased, as well as $\sigma(A)$. It was also accepted for 10 % v/v ethanol with a similar K_{12} value (log $K_{12} \approx -4.5$) and also in 30 % v/v DMF, but rejected in 50 % ethanol and 30 % methanol (see Table 4).

To be quite certain about the existence of this complex the absorbance – pH curves also for two higher c_R values (1×10^{-4} and 2×10^{-4} M TAMP) were measured, where one could expect more of this "dimer". In calculations on these data the species was not accepted and then rejected when treating series 2, 3, and 4 altogether.

A probable explanation of $R(RH)^-$ being accepted in some series is the complex formation of TAMP with some metal impurities in the ionic medium in spite of the presence of EDTA in most of the cases. And really, the best "improvement" in the U value after adding species $R(RH)^-$ was observed for H_2O, $C_{EDTA} = 0$ but no improvement was obtained for series with higher EDTA concentrations. We can therefore imagine, that the acceptance of the hypothetical reaction (B) with the equilibrium constant K_{12} (*cf.* Table 4)

$$R^- + RH \rightleftharpoons R(RH)^- \qquad (B)$$

is rather due to complex formation of TAMP with metal impurities, Me^{z+} such as

$$Me^{z+} + 2\ RH \rightleftharpoons MeR(RH)^{(z-1)} + H^+ \qquad (C)$$

$$\text{or } Me^{z+} + R^- + RH \rightleftharpoons MeR(RH)^{(z-1)+} \qquad (D)$$

The formation of mixed species containing EDTA cannot be excluded, of course. This interfering reaction was hindered by higher EDTA concentrations in the case of series 3 and 4, so that $R(RH)^-$ species was not accepted. The same can be true for the other media as well, thus we can eventually conclude that no "dimer" $R(RH)^-$ is formed during the experimental conditions used in this paper.

CONCLUSIONS

Three optically different species of TAMP exist in aqueous as well as in mixed solvents depending on the pH value. The first orangered species RH_2^+ with absorption maximum 495 – 520 mn predominates down to pH ≈ 1, the second form RH is yellow with maximum 465 – 475 nm, predominating in the pH range 1 – 7 and the violet-blue form R^- ($\lambda_{max} = 560 - 570$ nm) predominates above pH = 7. There are two isosbestic points on the absorption curves – one of them indicates deprotonation of RH_2^+ and the second the deprotonation of RH to yield R^- (see Table 6).

Absorption curves of the reagent measured in the pH region 0 – 11 and in concentrated sulphuric and perchloric acids were found to be in good agreement with Chromý and Sommer.[6] The values of λ_{max} which have been found for the species RH_2^+, RH, and R^- in different media are given in Table 5.

All methods for the determination of dissociation constants, described above, were applied only to the dissociation of TAMP (K_1) in all aqueous as well as in mixed solvents (except 20 and 40 % ethanol) because these data were obtained with an accuracy good enough to justify use of computer methods.

The dissociation constant of the protonated form of TAMP (K_2) was determined for aqueous solutions and for 30 % v/v ethanol by the graphical method by extrapolation of $\log \{(A - A_{01})/(A_{02} - A)\} = f(pH)$ plots for

Table 5. Summary of optical data of TAMP.

Form	Solvent	pH region	λ_{max} (nm) This paper	ε^a	λ_{max} (nm) Lit.	Ref.
RH_2^+	H_2O	1 [b]	510, 381	10 420	523, 380	6
	30 % CH_3OH		495, 382		372	1
	30 % DMF		493, 376			
RH	H_2O	1 – 7	467, 369	7.480	468, 370	6
	30 % CH_3OH		474, 370	8.550	462, 366	1
	30 % DMF		476, 370	8.750	481, 365	38
R^-	H_2O	7	559, 369	15 420	561, 370	6
	30 % CH_3OH		565, 368	15 520	562, 366	1
	30 % DMF		571, 370	16 510		
	10 % C_2H_5OH			15 840		
	50 % C_2H_5OH			17 070		

[a] $\varepsilon_{RH_2^+}$ given for 510 nm, ε_{RH} for 470 nm, ε_{R^-} for 560 nm.
[b] The ionic strength was varied in the range 0.10 – 1.20 (HNO_3 + 0.10 M KNO_3).

Table 6. Final values of the protonation constant of TAMP, determined by graphical method and final values for the dissociation constant as found by LETAGROP-SPEFO. A comparison with literature data.

Equilibrium	Solvent	λ Isosbestic point	This paper ($I=0.10(KNO_3)$	$-\log K$ 25.0°C	Lit.	Ref.
$RH_2^+ \rightleftharpoons RH + H^+$	H_2O	418 nm	0.00 ± 0.04[a]		0.03 ± 0.03	6
	30 % EtOH		0.12 ± 0.05[a]			
	30 % dioxane				0.3	8
$RH \rightleftharpoons R^- + H^+$	H_2O	493 nm	7.858 ± 0.009		8.13 ± 0.02	6
	30 % CH_3OH		8.159 ± 0.006			
	30 % DMF		8.386 ± 0.006			
	10 % EtOH		7.923 ± 0.009			
	20 % EtOH		8.18 ± 0.03[a]			
	30 % EtOH		8.328 ± 0.017			47
	40 % EtOH		8.56 ± 0.06[a]			
	50 % EtOH		8.925 ± 0.016			
	20 % dioxane				8.4	1
	20 % dioxane				8.2	39
	30 % dioxane				7.90	8
	50 % dioxane				9.18	3

[a] Determined by means of the graphical method only.

pH < 1.0. The values of $-\log K_2$ obtained are equal to 0.00 ± 0.04 and 0.12 ± 0.05, respectively. One can see that the protonation of TAMP becomes important only in rather strongly acidic solutions, *i.e.* below pH = 1.

The relevant values of absorption maxima λ_{max}, wavelength of isosbestic points λ_{isosb}, and final values of $-\log K_1$ and $-\log K_2$ and molar absorptivities of all species determined by LETAGROP-SPEFO procedure are listed in Tables 5 and 6.

It deserves to be mentioned that the small amount of DMF used to bring TAMP into solution in the various solvents has a negligible effect on the pK-values obtained.

The difference in pK between 0.1 % DMF solution and that obtained for pure H_2O (calculated from the difference of pK values obtained for H_2O and 30 % DMF in this paper) is about 0.002 pK which is much less than the experiment uncertainty.

From Tables 2 and 3 where the values of $-\log K$ and ε_i are listed one can see, that individual values determined by different procedures are in good agreement, irrespective of the different basic principles of the graphical and least squares method on one hand, and of LETAGROP-SPEFO on the other.

The program PRCEK is useful for the determination of equilibrium constants in cases, where only two absorbing species are present in solution in the pH interval studied. In more complicated cases, if three absorbing species are present, the problem can be solved by dividing the investigated pH interval into regions where only two species absorb. It is the same principle as that one used in solving simultaneous equilibria by graphical methods.

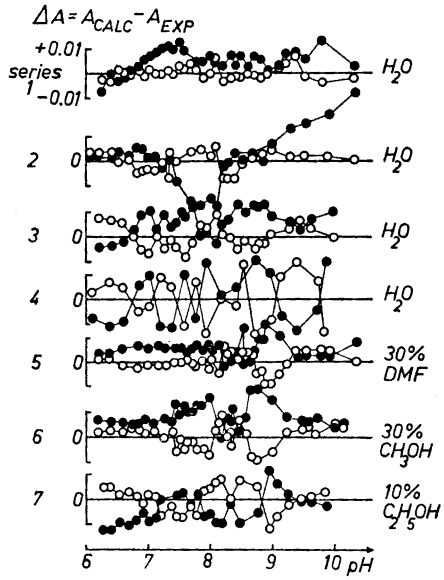

Fig. 3. The deviations $\Delta A = A_{calc} - A_{exp}$ at the arbitrarily chosen wavelength 560 nm as a function of pH for various mixed solvents, determined by PRCEK (open symbols) and LETAGROP-SPEFO (filled symbols). The conditions for each series are given in Table 1a. The data in this figure correspond to ~ 1/6 of all the experimental data.

As seen from Table 2 the program PRCEK gives error square sums about three times as big as LETAGROP. However, the difference in the constants obtained is so small that results with PRCEK are acceptable. The highest deviation between the pK value calculated by means of PRCEK and LETAGROP-SPEFO was for series 1, *i.e.* 0.015 logarithmic units, but the average absolute deviation for all the series was 0.005 logarithmic units, which is quite acceptable (see Table 2).

A comparison of the deviations $\Delta A = A_{calc} - A_{exp}$ calculated by both programs for various mixed solvents is given in Fig. 3 for one of the wavelengths. It may be seen that PRCEK gives slightly higher deviations, but for the present purpose this is not serious.

The final values for the dissociation constants of TAMP are given in Table 6 and are those calculated by LETAGROP-SPEFO, since they are taken as the "best" values obtainable from our experimental data.

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