Physicochemical Properties of a New Multicomponent Cosolvent System for the pK_a Determination of Poorly Soluble Pharmaceutical Compounds

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A mixture of cosolvents is described that significantly improves the solubility of most pharmaceutical compounds. The mixture consists of equal volumes of MeOH, 1,4-dioxane, and MeCN, thereby containing polar and nonpolar solvents, and is referred to as MDM (from MeOH, dioxane, and MeCN). MDM is mixed with H_2O until the required composition is reached. The utility of this system is that it enables analytical measurements to be performed on a wide range of compounds where measurements would be impaired in aqueous solution. We present the physicochemical characteristics of MDM/H_2O mixtures (density, dielectric constant, p_sK_w) and the principles of pK_a measurement in this solvent/H_2O mixture. We also present pK_a values in H_2O of several drug compounds determined from values measured in MDM/H_2O mixtures.

Introduction. – Modern techniques of drug discovery often produce molecules that are poorly soluble in H_2O. Insoluble molecules can be difficult to formulate into effective drugs, and a good deal of research is devoted to efforts to improve solubility without diminishing the efficacy of promising molecules. Poor aqueous solubility also creates problems for analytical chemists because many assays require the drugs to be in solution during measurement. Typical problems that occur when samples are not in solution are false positives or negatives in enzyme activity assays, anomalous dose–response curves and erratic pharmacokinetic profiles, poor mass balance in membrane permeation assays, and errors in physicochemical-property determinations such as lipophilicity and ionization. Many synthesized compounds can be dissolved in DMSO, and this solvent is used to prepare compound libraries. Deuterated DMSO is valuable as a solvent in NMR because it contains no protons. However, DMSO is not an ideal matrix for presenting samples to other instrumentation, because it has high UV absorbance, absorbs H_2O at room temperature, and causes big shifts in the pH scale. Analysts risk losing time and sample when seeking alternative solvents, and a more universal solvent would be useful.

Ionizable drugs are more H_2O-soluble when charged but may be insoluble when uncharged. The solubility of some unionized molecules can be enhanced by mixing solvents such as MeOH, dioxane, or MeCN with H_2O, but experience shows that not all compounds dissolve in any single solvent/H_2O mixture. In this paper, we characterize a

mixture of polar and nonpolar H_2O-miscible solvents consisting of equal volumes of MeOH, dioxane, and MeCN, referred to as MDM (from MeOH, dioxane and MeCN).

The solvation properties of MDM/H_2O have been evaluated by computer simulations based on molecular modeling *via* Monte Carlo and molecular-dynamics simulations, which found for a series of fluoroquinolones that H_2O mainly solvated the polar sites of the solutes, while dioxane was the most important organic component [1]. Besides dissolving fluoroquinolones, MDM has been shown to dissolve analytical quantities of a wide range of poorly soluble drugs, as shown in *Fig. 1*, which depicts the solubility of 40 predominantly poorly soluble compounds in 20 and 40% (*v*/*v*) MDM/H_2O at two concentrations (1 mM, required for potentiometric pK_a measurement, and 20 μM, required for pH/UV pK_a measurement). The 40% (*v*/*v*) MDM/H_2O mixture dissolved more than two thirds of the samples at 1 mM, while only one sample remained insoluble at 20 μM [2]. MDM also has good stability, low viscosity, low UV absorbance, and predictable effects on the pH scale.

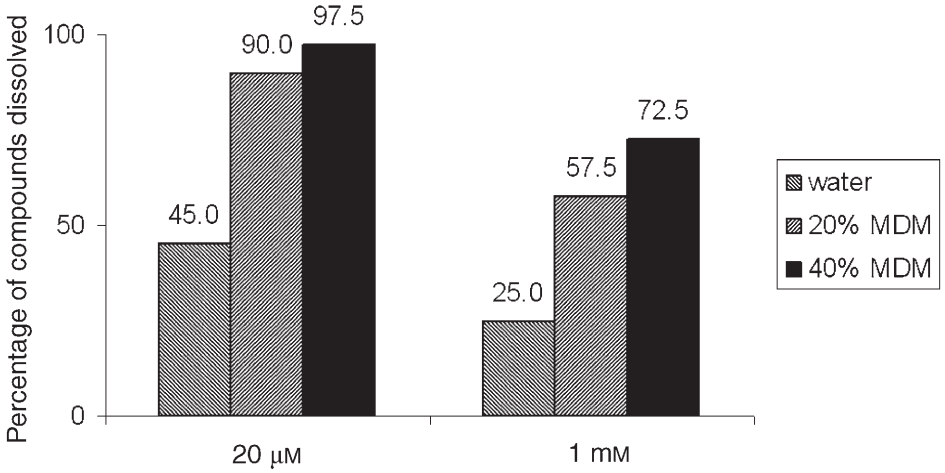

Fig. 1. *Improvement of the solubility of 40 drug samples in MDM/H_2O mixtures* (expressed as % of molecules dissolved in two anal. quantities: 1 mM and 20 μM concentrations). Compounds: 3,5-Dinitrobenzoic acid, acetaminophen, ampicillin, antipyrine, atenolol, buspirone·HCl, captopril, chlorpromazine·HCl, clioquinol, debrizoquine sulfate, diazepam, diphenoxylate·HCl, estradiol, furosemide, haloperidol, hexachlorophene, hydrochlorothiazide, ibuprofen, labetalol·HCl, mexiletine·HCl, naproxen Na, nifedipine, nitrazepam, oxytetracycline·HCl, phenylbutazone, piroxicam, prazosin·HCl, quinidine sulfate, salicylic acid, selegiline·HCl, sulfadimidine, plus eight molecules under development.

While MDM could be useful for other assays, this work was stimulated by the need to dissolve samples prior to measurement of their pK_a values. The measurement of pK_a requires correct reporting of experimental pH values. The pH scales in H_2O are known to shift in the presence of other solvents, but corrections can be made if physicochemical properties of the solutions including densities, dielectric constants,

and p_sK_w[1]) (autoprotolysis) values are taken into account [3]. This paper reports on the determination of these physicochemical properties of MDM/H_2O mixtures, and illustrates their use for obtaining reliable pK_a values in H_2O of three acidic and three basic drug molecules. A validation study comprising pK_a measurement in MDM/H_2O of 50 drug molecules, and a study reporting the use of MDM to dissolve meclizine for pK_a and solubility measurement have been published recently [4][5]; both studies make use of the physicochemical properties reported here.

Results and Discussion. – *Characterization of MDM Mixtures.* Certain physicochemical properties of MDM/H_2O mixtures, namely density, dielectric constant, and autoprotolysis constant p_sK_w[1]), were measured to facilitate the general use of MDM/H_2O for pK_a measurements.

The densities of standard MDM/H_2O mixtures were measured to be able to express the MDM content of any MDM/H_2O mixture prepared by volumetric dilution in terms of mass-% MDM. This also facilitates the calculation of H_2O content in MDM/H_2O mixtures for use in the *Yasuda–Shedlovsky* (*YS*) extrapolation method for determining pK_a values in H_2O (see below). The values of the relative densities are indicated in *Table 1*. As a comparison, the relative density of 100% (*v*/*v*) MeOH is 0.787 g/cm^3, that of 100% (*v*/*v*) dioxane 1.028 g/cm^3, and that of 100% (*v*/*v*) MeCN 0.761 g/cm^3.

Table 1. *Relative Densities and Dielectric Constants of Solvent/H_2O Mixtures.* Values calculated in with the *Sirius* RefinementPro™ software by interpolation from published data.

Percent cosolvent (*v*/*v*)	Relative density [g/cm^3] of MDM/H_2O	Dielectric constant			
		MDM/H_2O[a])	MeOH/H_2O[b])	dioxane/H_2O[c])	MeCN/H_2O[d])
10%	0.993 ± 0.001	–			
20%	0.987 ± 0.001	70.2 ± 0.1	71.4	60.7	72.2
30%	0.979 ± 0.001	64.6 ± 0.8	67.6	51.3	68.1
40%	0.971 ± 0.001	59.0 ± 0.7	63.5	41.9	63.4
50%	0.960 ± 0.001	53.5 ± 0.9	59.1	32.9	58.5
60%	0.949 ± 0.001	47.9 ± 0.4	54.3	24.6	53.5
100%	0.871 ± 0.001	–	31.7	1.8	43.2

[a]) This work. [b]) [6]. [c]) [7]. [d]) [8].

Dielectric constants of MDM/H_2O and its components in different concentrations were measured to use them in *YS* extrapolations of p_sK_a[1]) values measured in MDM/H_2O mixtures. The method used involves measurements of the capacity of a condenser filled with the dielectric medium. Thus, the capacity of the dielectrometer's condenser (measured in vacuum between the plates of the condenser) increases when filled with dielectric medium according to *Eqn. 1*, where ε_r is the dielectric constant of the dielectric medium, C_0 is the electric capacity in vacuum, and C is the electric capacity in the medium. The C_0 is effectively equivalent to the capacity measured in CO_2-free air because the dielectric constant of CO_2-free air is 1.00058 at 0° and 1 atm pressure. The

[1]) The preceding subscript (or superscript) s refers to a solvent mixture or a solvent different from H_2O.

relationship of *Eqn. 2* describes the dielectric constant of MDM/H_2O mixtures, where C_{MDM} and C_{water} are the capacities of condensers filled with MDM or distilled H_2O, and ε_{MDM} and ε_{water} are the dielectric constants of MDM/H_2O mixture or of distilled H_2O. The dielectric constant of distilled H_2O is well-known ($\varepsilon_{water} = 78.3$), so the dielectric constant of MDM/H_2O mixtures can be calculated from the measured capacities. The results are also summarized in *Table 1*. The measured dielectric constant of MDM/H_2O mixtures is higher than the composite value derived from averaging the individual cosolvent dielectric values for all MDM/H_2O ratios studied showing a common behavior of aqueous/organic mixtures [9].

$$C = \varepsilon_r \, C_0 \tag{1}$$

$$\varepsilon_{MDM} = (\varepsilon_{water} - 1)\frac{C_{MDM} - C_0}{C_{water} - C_0} + 1 \tag{2}$$

The autoprotolysis constants of MDM/H_2O (p_sK_w[1]) values) were measured to be able to calculate j_{OH} values used in the conversion of pH (activity pH values) to p[H] (concentration pH values) of MDM/H_2O mixtures. The principle of the method is to calculate p_sK_w from independent measurements of the values of the standard acidic and basic potentials (E^0_a, E^0_b) in *Eqn. 3*, where g is the *Nernst* constant. Values for p_sK_w were determined by the *Gran* method, with the functions of *Eqn. 4* in acidic medium, and of *Eqn. 5* in basic medium. In *Eqns. 4* and *5*, E is the measured potential, and $j_{H_2S^+}$ and j_{S^-} are the contributions to the liquid-junction potentials from the lyonium (H_2S^+) and the lyate (S^-), respectively. The activities (a_i) are related to molarity (c_i) through the corresponding activity coefficient γ_i by *Eqn. 6*.

$$E^0_b = E^0_a - g \; p_sK_w \tag{3}$$

$$E = E^0_a + j_{H_2S^+} a_{H_2S^+} - g \; pH \tag{4}$$

$$E = E^0_b - j_{S^-} a_{S^-} + g \; pS \tag{5}$$

$$a_i = c_i \, \gamma_i \tag{6}$$

Activity coefficients were calculated by using the *Debye–Hückel* equation (*Eqn. 7*), where z is the charge of the ion, I is the ionic strength, A and B are two fundamental solvent constants, and a_0 stands for the ion-size parameter, which is assigned a value fixed by the *Bates–Guggenheim* convention [10] extended to solvents of relatively moderate permittivities. ${}^w\varepsilon$, ${}^w\rho$, ${}^s\varepsilon$[1]), and ${}^s\rho$[1]) denote the dielectric constants and the densities of H_2O and of the MDM/H_2O mixture. In *Table 2*, calculated a_0B and A values are compiled for different MDM/H_2O mixtures.

$$\log \gamma_i = -\frac{A z^2 \sqrt{I}}{1 + a_0 B \sqrt{I}} \tag{7}$$

$$a_0 B = 1.5 \sqrt{\frac{{}^w\varepsilon \, {}^s\rho}{{}^s\varepsilon \, {}^w\rho}} \tag{8}$$

Table 2. *Calculated* a_0B *and* A *Values and Experimentally Determined* p_sK_w *Values* vs. *Mass-% MDM in the Solvent Mixture*

Mass-% MDM	a_0B	A	p_sK_w (s.d.)[a])
0	1.520	0.532	14.00
8.8	1.532	0.549	14.03 (0.06)
17.6	1.576	0.603	14.14 (0.02)
26.7	1.631	0.675	14.39 (0.03)
35.9	1.697	0.771	14.71 (0.05)
45.4	1.772	0.891	14.93 (0.04)
55.1	1.854	1.040	15.20 (0.03)

[a]) s.d. = Standard deviation.

$$A = \frac{1.8246 \cdot 10^6}{\sqrt{({}^s\varepsilon T)^3}} \tag{9}$$

Figs. 2,a and *b* show the plots of *Gran* functions of a 20% (*v*/*v*) MDM/H_2O mixture. In *Fig. 2,a*, the plot reveals a straight line with a slope value near to zero. Therefore, the liquid-junction potential E_j is negligible in acidic medium. *Fig. 2,b*, however, exhibits a positive slope value of *ca.* 400 mV, meaning that the liquid-junction potential E_j must be considered in basic media. *Table 2* shows the experimentally determined p_sK_w values and the A and a_0B coefficients for the *Debye–Hückel* corrections.

The change in p_sK_w values with the mass fraction MDM (m_{MDM}) can be fitted to the polynomial *Eqn. 10*. *Eqn. 10* shows that p_sK_w increases with increasing MDM content, demonstrating that the pH scale is extended and shifted in MDM/H_2O mixtures as compared to aqueous solutions. For example, pK_w in pure H_2O is 13.997 at 25° and neutral pH of H_2O (where $[H^+] = [OH^-]$) is at pH 7, whereas in 50 mass-% MDM/H_2O, where $p_sK_w = 15.02$, neutral pH is at 7.51. This extended scale is evident from blank titrations in pure H_2O and blank titrations in MDM/H_2O as shown in *Fig. 3*. For example, the slope of the titration curve with respect to titrant addition shows that the steepest regions of the titration curve occur between pH *ca.* 3.4 and 10.3 under aqueous conditions, whereas in 50 mass-% MDM/H_2O the equivalent steepest regions occur between pH *ca.* 3.5 and 11.3. Indeed, the whole pH scale is shifted upwards and extended, and the unbuffered pH region covers an additional extra pH unit at high MDM content. In comparison, the individual cosolvent components have p_sK_w values of 14.18, 15.73, and 15.74 at 50 mass-% MeOH/H_2O, dioxane/H_2O, and MeCN/H_2O, respectively. Hence, the MDM displays almost composite properties. The largest difference from composite properties occurs at *ca.* 20 mass-% cosolvent where the p_sK_w is 14.19, 14.10, 14.57, and 14.56 for MDM, MeOH, dioxane, and MeCN respectively.

$$p_sK_w = -10.56\ m_{MDM}^3 + 10.60\ m_{MDM}^2 - 0.62\ m_{MDM} + 14.00) \tag{10}$$

$$r^2 = 0.998;\ \text{s.d.} = 0.03;\ n = 7$$

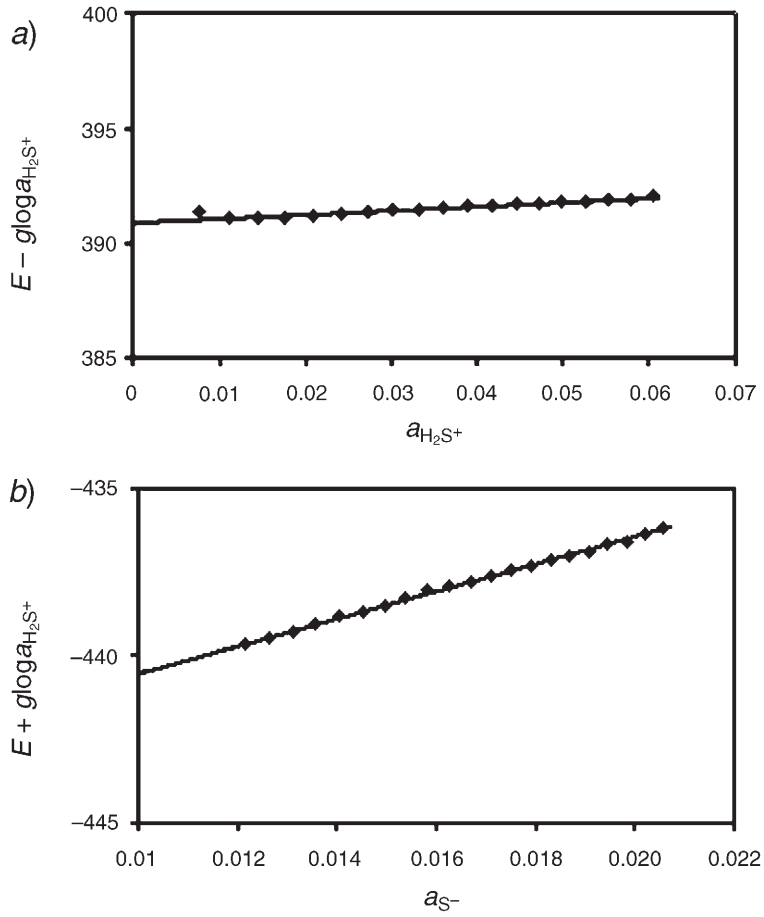

Fig. 2. Gran-*function plots:* a) $(E - g\ log\ a_{H_2S^+})$ vs. $a_{H_2S^+}$, *and* b) $(E + g\ log\ a_{S^-})$ vs. a_{S^-} *at 20%* (*v*/*v*) *MDM*/H_2O

Titrations. To calibrate the potentiometric system, the measured pH (= pH_m) was calibrated to the p[H] (= $-\log[H^+]$) by performing a weighted linear least-squares fit of the form of *Eqn. 11*, where *S* is the electrode slope compared to the *Nernst* slope, and α mainly corresponds to the negative logarithm of the activity of $[H^+]$ at the working temperature and ionic strength. The terms j_H and j_{OH} account for electrode-junction effects at low and high pH, respectively. To calculate j_{OH} values, it is necessary to have independently determined the p_sK_w autoprotolysis values in MDM/H_2O mixtures. The variation in values of α and *S* with MDM content at 25° is shown in *Figs. 4*, *a* and *b*. In these graphs, the values of α and *S* were determined at different mass-% MDM by fitting the experimental pH/ml data from each blank titration to computed values for p[H], α, *S*, j_H, and j_{OH}. Polynomial expressions were then fitted to the α and *S vs.* the mass-% MDM, as described elsewhere [11]. Values of α and *S* appropriate for other mass-% MDM can then be determined by interpolation.

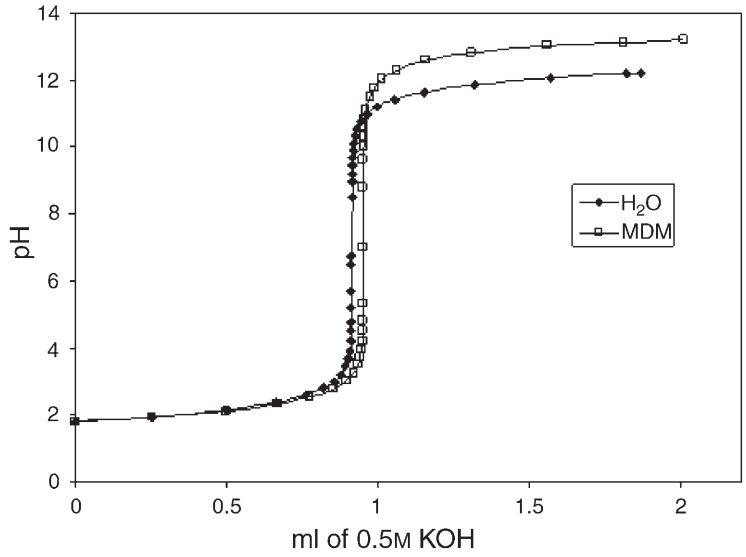

Fig. 3. *Blank titration in pure H_2O* (◆) *and blank titration in 50 mass-% MDM* (□)

$$pH_m = \alpha + S\ p[H] + j_H\ [H^+] + j_{OH}\ [OH^-] \quad (11)$$

Values for α, S, j_H, and j_{OH} define the calibration of a given pH electrode. They are referred to as 'Four-Plus' values, and are determined experimentally by a blank titration, in which a measured volume of aqueous electrolyte (*e.g.*, 20 ml of 0.15M KCl) is titrated between pH 1.8 and 12.2 with 0.5M HCl and 0.5M KOH. A blank titration in H_2O is generally performed before titrations for pK_a measurements. For p_sK_a measurements in solvent/H_2O mixtures, the 'Four-Plus' values from an aqueous blank can be converted into new values appropriate for each ratio solvent/H_2O. This conversion is done by multiplying each 'Four-Plus' value in H_2O by a correction factor. The correction factors are characteristic of a given solvent [3], and must first be determined from the results of blank titrations done in solvent/H_2O mixtures. Determination of the changes in 'Four-Plus' values as a function of solvent is a tedious necessity, but once they have been determined, they do not change and may be applied thereafter for all titrations in mixtures of H_2O with that solvent.

As shown in *Fig. 4*, the α values determined in MDM/H_2O mixtures decrease to a minimum value of 0.0225 at *ca.* 15 mass-% MDM followed by a rise to a maximum value of 0.144 at *ca.* 45 mass-% MDM. These values correspond to activity coefficients of 0.95 and 0.72, respectively. Hence, hydrogen-ion activity increases as high as *ca.* 95% at 15 mass-% MDM, and then the activity decreases to *ca.* 72% at 45 mass-% MDM. Conversely, the slope of the electrode when compared to Nernstian slope shows the opposite behavior, increasing to a maximum at *ca.* 15 mass-% MDM and reaching a minimum at *ca.* 45 mass-% MDM.

The effect of the 'Four-Plus' electrode parameters can be demonstrated by considering how the calculation of the p_sK_a would be changed if only measured pH

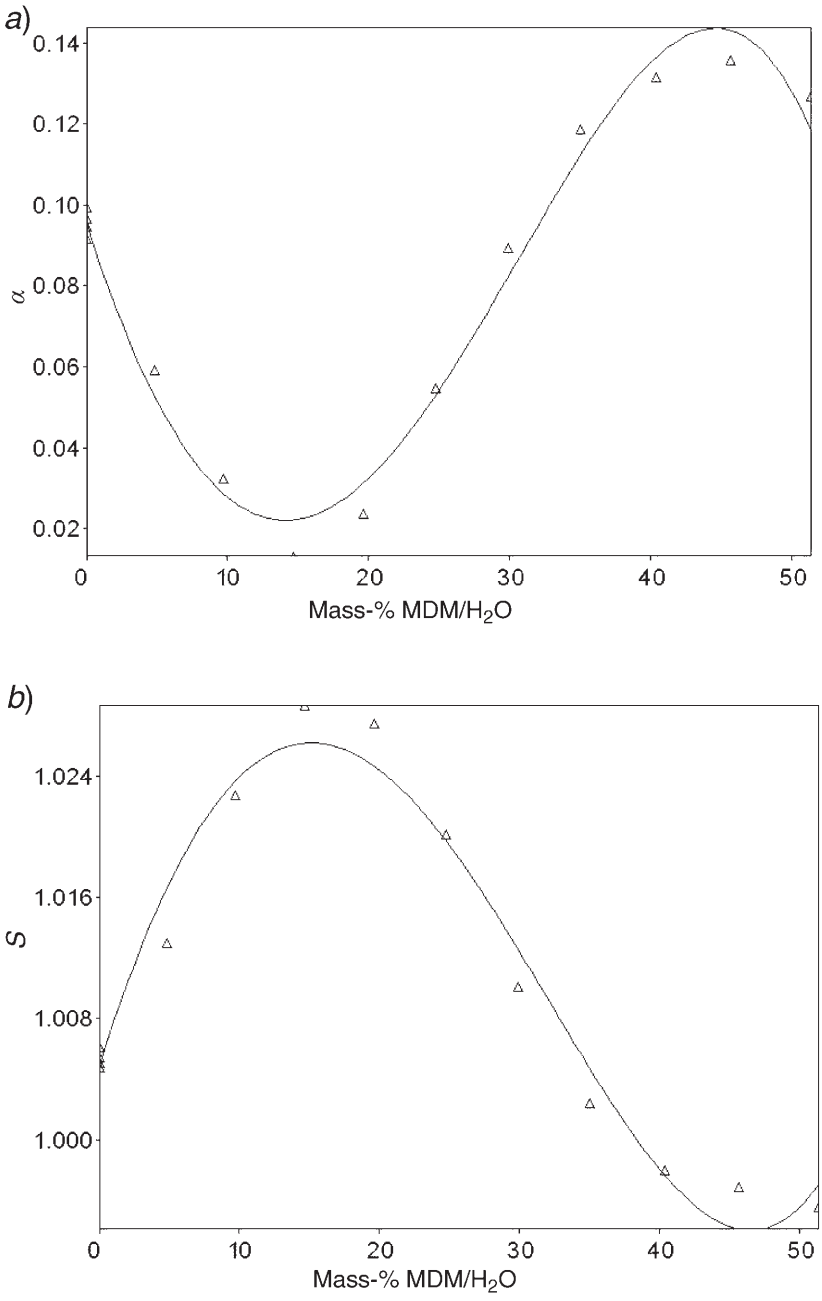

Fig. 4. *Electrode calibration factors* (α, slope) *in MDM/H_2O mixtures*

values were used. If measured pH values produced a result where the apparent $p_sK_a = 7.00$, then this would translate to a concentration-based p_sK_a of 6.80 at 15 mass-% MDM, whilst at 45 mass-% MDM, the concentration-based p_sK_a would equal 6.90. These differences between hydrogen-ion activity *vs.* hydrogen-ion concentration as a function of pH are revealed most readily in *Fig. 5*, which displays pH – p[H] for varying MDM contents. The largest difference, greater than 0.25 pH units, occurs above pH 11 at 15 mass-% MDM. Hence, it is important to apply MDM 'Four-Plus' calibration parameters to enable the conversion of pH to concentration p[H] for a reliable determination of concentration-based ionization constants (p_sK_a values) in the presence of MDM.

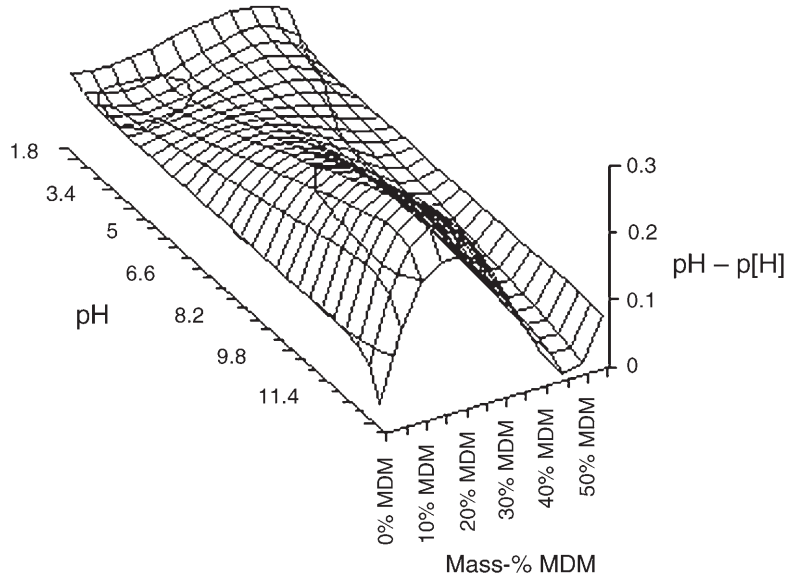

Fig. 5. *Variation in activity* vs. *concentration pH for changing MDM content*

Acidic Dissociation Constants of Drugs. The p_sK_a values in MDM/H_2O mixtures for the six compounds shown in *Fig. 6* (amiodarone, bifonazole, diclofenac, phenazopyridine, sulfasalazine, and tartaric acid) are shown in *Table 3*. As expected [3], the p_sK_a values of the acidic compounds (tartaric acid, diclofenac, sulfasalazine) increase with MDM content, whereas they decrease for bases (amiodarone, bifonazole, and phenazopyridine). The factors that contribute to the variation of p_sK_a when a solute is transferred from water (w) to another pure or mixed solvent (s) are summarized in the *Brönsted* equation [13] (*Eqn. 12*), where $p_{vac}K_{H_3O^+}$ and $p_{vac}K_{HS^+}$ indicate the intrinsic acidities of the protonated H_2O and solvent S (= MDM/H_2O mixture) in vacuum. The term $[e^2(z-1)/2.303rkT](1/^s\varepsilon - 1/^w\varepsilon)$, where e is the electron charge and kT the energy of thermal agitation, stands for the differences of the electrostatic interactions between the ions present in the solutions in solvent S and in H_2O, which depends on the charge z of the acid, the static dielectric constants of the two solvents $^s\varepsilon$

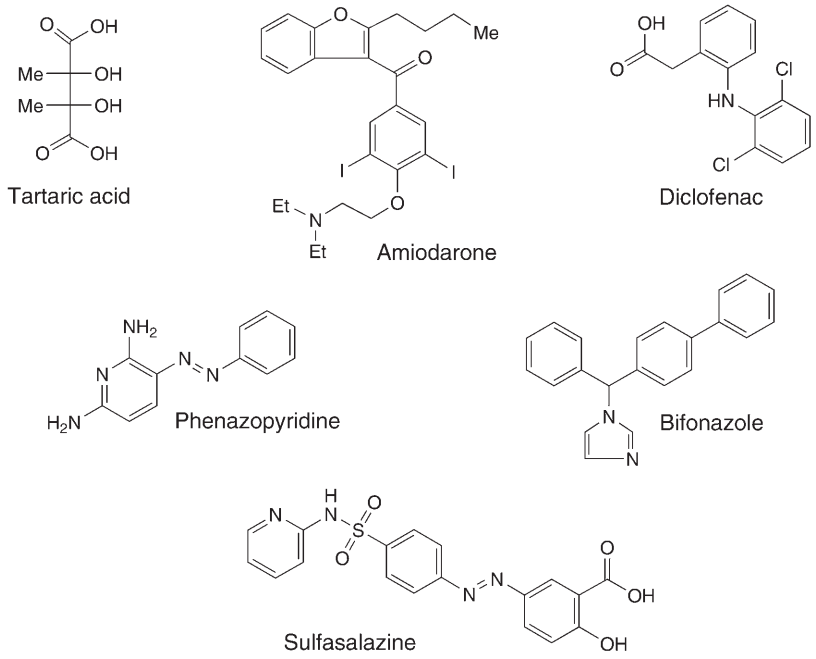

Fig. 6. *Structures of the six molecules used in this study*

and ${}^{w}\varepsilon$, and on the radius of the ions r. The last term of *Eqn. 12* stands for the differences in the energies of specific solvation of the acid in the two solvents.

$$\Delta pK_a = {}_{s}pK_a - {}_{w}pK_a$$
$$= {}_{vac}pK_{H_3O^+} - {}_{vac}pK_{HS^+} - \frac{e^2(z-1)}{2.303rkT}\left(\frac{1}{{}^{s}\varepsilon} - \frac{1}{{}^{w}\varepsilon}\right) - \frac{(\Sigma_s G_{solv} - \Sigma_w G_{solv})}{2.303RT} \qquad (12)$$

The different behavior of cationic acids (*e.g.*, phenazopyridine) and neutral (*e.g.*, diclofenac) or anionic (*e.g.*, hydrogen tartrate) acids comes from the different contributions of the electrostatic term of *Eqn. 12*. For a cationic acid, $z = 1$ and the electrostatic term cancels out. Therefore, ΔpK_a depends only on the differences between the acidities of the protonated H_2O and MDM/H_2O mixture and on the differences between the specific solvation terms, which in general are small and negative compared with the electrostatic contribution (which is positive for $z \leq 0$), and explain the pK_a variation of neutral and anionic acids. As expected, in all instances, ΔpK_a increases in absolute value with the MDM contents in the solvent [14][15].

The *YS* extrapolation is widely accepted for extrapolating ${}_{s}pK_a$ values measured in several different solvent/H_2O mixtures to pK_a values in H_2O. In *YS* extrapolations, the ${}_{s}pK_a$ determined at a given solvent/H_2O ratio is added to the log of the concentration of water [H_2O] at that ratio, and the sum is plotted against the inverse of the dielectric

Table 3. *Measured p_sK_a Values of the Study Compounds in MDM Cosolvents*

	Bifonazole		Phenazopyridine		Sulfasalazine				Amiodarone		Diclofenac		Tartaric acid		
	p_sK_a	mass-% MDM	p_sK_a	mass-% MDM	p_sK_{a3}	p_sK_{a2}	p_sK_{a1}	mass-% MDM	p_sK_a	mass-% MDM	p_sK_a	mass-% MDM	p_sK_{a2}	p_sK_{a1}	mass-% MDM
	5.47	48.7	4.66	44.5	13.03	8.61	3.04	46.8	8.12	56.3	5.43	44.5	4.75	3.72	50.8
	5.56	43.1	4.73	36.1	12.67	8.50	2.88	39.7	8.27	48.3	5.03	36.1	4.54	3.51	39.5
	5.59	35.9	4.80	28.2	12.26	8.36	2.72	32.3	8.33	44.8	4.77	28.4	4.47	3.45	35.6
	5.73	25.3	4.85	23.3	11.91	8.23	2.60	24.4	8.36	42.4	4.65	23.4	4.33	3.29	29.0
	5.88	17.5	4.90	15.3	11.46	8.10	2.46	16.3	8.47	37.2	4.39	15.3	4.26	3.22	24.8
													4.18	3.11	20.4
A of *Eqn. 13*	− 124.6		− 103.4		287.4	64.7	78.4		− 135.2		187.4		71.1	73.7	
B of *Eqn. 13*	9.263		8.008		9.135	8.855	3.013		12.275		3.436		4.785	3.708	
r^{2a})	0.980		0.998		0.993	0.991	0.998		0.995		0.992		0.987	0.958	
pK_a[b])	5.92 ± 0.04		4.94 ± 0.01		11.06 ± 0.05	7.94 ± 0.01	2.27 ± 0.01		8.80 ± 0.02		4.09 ± 0.03		3.96 ± 0.02	2.91 ± 0.05	
pK_a[c])	6.06[d])		5.07[d])		10.89[d])	8.00[d])	2.35[d])		8.73[d])[e])		3.99 [12]		3.87[d])	2.92[d])	

[a]) r^2 of the *YS* extrapolation. [b]) Extrapolated pK_a in H_2O. [c]) Alternative pK_a in H_2O. [d]) Measured in-house at *Sirius* by conventional pH-metric technique. [e]) *Yasuda–Shedlovsky* extrapolation from MeOH/H_2O solutions.

constant ε of the given mixture, as described in *Eqn. 13*, where A and B are the slope and intercept of the plot, respectively. Points are extrapolated linearly to the inverse of the dielectric constant of H_2O. The log of the concentration of H_2O in H_2O (log 55.5M) is subtracted from the intercept, and the result is taken as the pK_a value in H_2O. This method has been validated for several solvent/H_2O systems [3][16]. *Table 3* gives the equations and regression coefficients for the six compounds in this study, and extrapolations are displayed in *Figs. 7,a* and *b*.

$$p_sK_a + \log [H_2O] = A/\varepsilon + B \tag{13}$$

It has been reported that *YS* extrapolations to aqueous conditions are linear for many solvents when ε values greater than 50 are used [16]. This would allow for p_sK_a measurements in MDM/H_2O with MDM contents up to 53 mass-%. The only compound where a higher MDM content was required was amiodarone. For this compound, the *YS* extrapolation from 56 mass-% MDM/H_2O was still linear, with $r^2 = 0.995$, and the extrapolated value was in good agreement with results obtained in MeOH/H_2O.

The pK_a values determined by *YS* extrapolation agree well with reported pK_a values in H_2O for all compounds. The average error is 0.10 pK_a units between the two sets of values, with a maximum deviation of 0.17 pK_a units for the highest pK_a of sulfasalazine. Amiodarone precipitated at all MDM contents below 37 mass-%. Nevertheless, the long-range extrapolation agreed with a result extrapolated from MeOH/H_2O solutions. Tartaric acid was analyzed pH-metrically at millimolar concentrations; all other samples were analyzed by UV spectroscopy at concentrations of *ca.* $3 \cdot 10^{-5}$ M. Solubility was adequate at all ratios of MDM apart from amiodarone described above. Intrinsic solubilities in H_2O of 0.9 μg/ml, 200 ng/ml, 5 ng/ml, and 12 μg/ml are reported for diclofenac [17], sulfasalazine [18], amiodarone [19], and phenazopyridine [19], respectively. Bifonazole is estimated to have an intrinsic solubility between 100 and 400 ng/ml. Solubility is found to increase sufficiently above 15 mass-% MDM/H_2O, allowing analysis of all the compounds, except for amiodarone. Tartaric acid is a H_2O-soluble compound although its potassium salt is known to be poorly soluble. It was analyzed pH-metrically at millimolar concentrations to illustrate the utility of the technique for polar molecules.

The magnitude of the slopes in the *YS* extrapolations may also provide information on the solvating ability of MDM for various compound classes. The slopes (A) for the bases are fairly consistent between -103 to -135 corresponding, *e.g.*, to a decrease in p_sK_a of 0.08 to 0.13 units between 20 and 30 mass-% MDM/H_2O. There is more variation for the acids; the slopes for the first two pK_a values of sulfasalazine and the tartaric acid pK_a values are quite low ($A = +65$ to $+78$) corresponding to increases in p_sK_a of 0.15 to 0.17 units between 20 and 30 mass-% MDM/H_2O, whereas for diclofenac, $A = +187$ and Δp_sK_a (20–30 mass-% MDM/H_2O) = 0.32 units, and for p_sK_{a3} of sulfasalazine, $A = +287$ and Δp_sK_a(20–30 mass-% MDM/H_2O) = 0.46 units. The p_sK_{a3} for sulfasalazine corresponds to the transition from a charge of -2 to -3, while the diclofenac p_sK_a refers to the change from the neutral species to the -1 charge state and is more easily compared to the monoprotic bases and p_sK_{a1} for the acids (with charge transitions from 0 to ± 1). It has been reported that (in MeOH) the slopes are

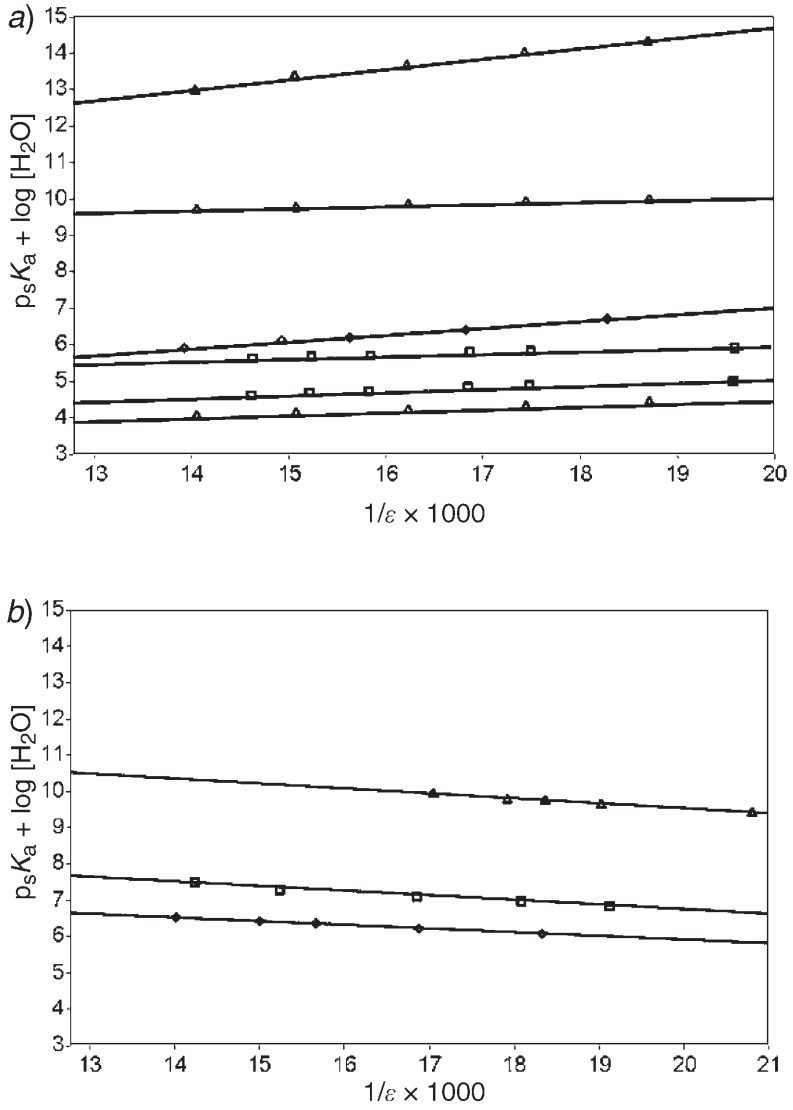

Fig. 7. a) Yasuda–Shedlovsky *extrapolations for the acidic compounds.* △, Sulfasalazine (3 pK_as); □, tartaric acid (2 pK_as); ◇, diclofenac (1 pK_a). b) Yasuda–Shedlovsky *extrapolations for the basic compounds.* △, Amiodarone (1 pK_a); □, bifonazole (1 pK_a); ◇, phenazopyridine (1 pK_a).

inversely proportional to the average ionic diameter of the solvated molecule [20]. The larger slope for diclofenac may indicate a decreased ability for solvation in MDM of this compound compared to the others. Nevertheless, the solubility in MDM/H_2O is enhanced sufficiently for easy analysis of diclofenac.

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Experimental Part

1. *General.* 1.1. *Chemicals.* p_sK_w *Measurements:* MeOH, MeCN, and 1,4-dioxane were HPLC grade from *Merck* (Darmstadt, Germany). *Titrisol®* KOH and 25% HCl soln. were from *Merck* (Darmstadt, Germany). Potassium hydrogen phthalate and *Tris* (=2-amino-2-(hydroxymethyl)propane-1,3-diol) were purchased from *Sigma–Aldrich* (Steinheim, Germany). Deionized H_2O (*Milli-Q* deionizer, *Millipore*, Bedford, MA, USA) was used to prepare the solvent mixture. HCl and KOH were standardized by titration against the primary standards *Tris* and potassium hydrogen phthalate, resp. *pH Electrode Calibration and* p_sK_a *Determination:* KCl (anal. grade) was from *SureChem*, 0.5M HCl was from *Aldrich*, and 0.5M KOH was made from an ampoule supplied by *Fisher Scientific*. Deionized H_2O of resistivity $>10^{14}\,\Omega$ cm was used to prepare all solns. Amiodarone, bifonazole, diclofenac, phenazopyridine, sulfasalazine, and tartaric acid were obtained from *Sigma–Aldrich*. *Density and Dielectric Constant Measurement:* MeOH, 1,4-dioxane, and MeCN (all HPLC grade) were obtained from *Merck*.

1.2. *Apparatus.* Densities were measured with a *43320* pycnometer of 25 ml volume (*Blaubrand*, Germany). Dielectric constants were measured with an *OH-301-Universal* dielectrometer (*Radelkis*, Hungary) at constant temp. ($25\pm0.1°$). For p_sK_w determinations, measurements were made with a *Crison micropH-2002* potentiometer with a precision of ±0.1 mV (±0.002 pH units) by using a *Sirius* Ag/AgCl pH electrode, accurate volume addition being made with a *Metrohm 665-Dosimat* autoburette controlled by the VALORA program [21]. All the experiments were performed at 25° under efficient stirring with a magnetic stirrer. The temp. was controlled by circulation of water through a jacket, from a thermostated bath and maintained within $\pm0.1°$. For p_sK_a determinations, titrations were done with a *Sirius GLpKa*™ titrator fitted with a combination Ag/AgCl pH electrode. The instrument was controlled and results were calculated with the *Sirius* RefinementPro™ software. Measurements of pK_a values by the pH/UV technique were done with the *Sirius D-PAS*™ UV spectrometer attachment for *GLpKa*. The *D-PAS* was fitted with a bifurcated fiber optic dip probe of path length 1 cm (*Hellma*, UK). A temp. probe monitored the temp. during the course of the titration. An overhead stirrer was connected to a motor whose speed of rotation was controlled by the instrument. Narrow polyimide-clad quartz capillary (0.5 mm inside diameter) tubes were connected to precision dispensers that were capable of delivering small, reproducible aliquots of liquid of known volume. All titrations were done in 0.15M KCl under Ar at $25\pm1°$, with standardized 0.5M HCl and 0.5M KOH.

2. *Procedures.* 2.1. *Preparation of MDM/*H_2O *Mixtures.* The mixtures were prepared by mixing measured volumes of aq. 0.15M KCl with measured volumes of MDM soln., prepared by mixing equal volumes of the three solvents MeOH, dioxane, and MeCN, and diluting this mixture with an equal volume of H_2O together with a weighed amount of KCl sufficient to create a solvent/H_2O mixture that was 0.15M KCl.

2.2. *Determination of Densities.* Densities were measured at MDM concentrations of 10, 20, 30, 40, 50, 60, and 100% (*v*/*v*) MDM/H_2O. Three parallel measurements were carried out for each cosolvent system at $25\pm0.1°$. This method is described in the Hungarian Pharmacopoeia [22] and European Pharmacopoeia [23].

2.3. *Determination of Dielectric Constants.* Three parallel measurements were carried out for MDM concentrations of 20, 30, 40, 50, and 60 % (*v*/*v*) MDM/H_2O systems.

2.4. *Determination of the Autoprotolysis* (p_sK_w) *Constants.* The pH electrode output was measured in 25 ml of MDM/H_2O mixtures of 10, 20, 30, 40, 50, and 60 % (*v*/*v*) MDM. Acidic potentials were obtained by successive additions of 0.1 ml (from 0.1 ml to 2.0 ml) of 2M and 1M HCl to each MDM/H_2O mixture. Likewise, basic potentials were obtained by successive additions of 0.2 ml (from 4.0 ml to 8.0 ml) of 0.1M KOH to each MDM/H_2O mixture. At least two addition series were performed at each concentration (HCl and KOH) and for each solvent percentage.

2.5. *Determination of pH Electrode 'Four-Plus' Values in MDM/H_2O.* Blank titrations of MDM/H_2O mixtures containing 0–50 mass-% MDM were performed from pH 1.8–12.2 without any sample present. The variation in values of α and S with MDM content at 25° is shown in *Figs. 4*,*a* and *b*. In these graphs, the values of α and S were determined at different percentages of MDM by fitting the experimental pH/ml data from each blank titration to computed values for p[H], α, S, j_H, and j_{OH}. Polynomial expressions were then fitted to the α and S *vs.* percentage of MDM, as described elsewhere [11]. Values of α and S appropriate for other percentages of MDM can then be determined by interpolation.

2.6. *Measurement of Sample p_sK_a Values in MDM/H_2O Mixtures.* The p_sK_a values of several insoluble samples were measured in MDM/H_2O mixtures, pH-metrically by using the *GLpKa* alone, or spectrophotometrically by using the *GLpKa* in combination with the *D-PAS* spectrophotometer. All titrations were performed under Ar at 25 ± 1°. In an automated pH-metric titration, the pH of a soln. was adjusted by adding acid or base until the sample was fully ionized, and then titrated with base or acid until the sample was fully unionized. The pH of the soln. was monitored with the pH electrode and corrected by the calibration procedure described, and p_sK_a values were determined by using mass- and charge-balance equations described previously [24][25]. A sample concentration of at least $5 \cdot 10^{-4}$ M was required. Evidence for precipitation of samples was established by using the fiber-optic probe to detect light scattering.

In the hybrid pH-metric/UV method, multi-wavelength UV absorbance of the sample soln. was monitored throughout the titration *in situ* by using the fiber-optic probe. With this method, significantly smaller sample concentrations are required for p_sK_a measurement (10^{-5} M or below). Samples must have a chromophore, and the absorbance must change as a function of ionization. The p_sK_a values were calculated by a technique based on target factor analysis (TFA) described previously [26][27]. All calculations were made with the RefinementPro™ software.

3. *Stability of MDM Mixtures.* MeCN is known to decompose to ammonia at high pH, while 1,4-dioxane is known to decompose slowly, forming peroxides. The highest pH used in the autoprotolysis studies was *ca.* 12.4, corresponding to 0.025M KOH, and there was no olfactory evidence of decomposition to ammonia; moreover, the solns. were freshly prepared and used immediately before decomposition could occur. In the electrode calibration titrations and sample p_sK_a titrations, the MDM/H_2O solns. were at high pH for only a few minutes during each titration. Again there was no olfactory evidence of decomposition to ammonia over this short period. As an additional check on stability, UV spectra and relative permittivity were recorded for freshly prepared solns. of 20 and 60% (*v*/*v*) MDM/H_2O. The solns. were then divided. Two were kept under cool, dark conditions, and two under normal laboratory-bench circumstances for a week, after which the UV spectra and relative permittivity were again measured. No changes in the UV spectra were observed in either case. No significant changes in relative permittivity were observed: 20% (*v*/*v*) MDM/H_2O: day 1 $\varepsilon = 70.2$, and day 7 $\varepsilon = 70.2$ (cool, dark) and 70.4 (lab bench); 60% MDM/H_2O: day 1 $\varepsilon = 47.9$, and day 7 $\varepsilon = 48.1$ (cool, dark) and 47.8 (lab bench). The results of the shelf-life stability tests suggest that 20 and 60% (*v*/*v*) MDM/H_2O can be kept without changing at least for a week.

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