

Solubility of Uric Acid in Salt Solutions and Artificial Urine

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Summary. The solubility of anhydrous uric acid was measured at 37.0°C in different salt solutions (ionic strength: $0.300 \text{ mol} \cdot \text{dm}^{-3}$) occasionally containing organic compounds and in standard reference artificial urine. The results did not significantly differ from the solubilities obtained previously in $0.150 \text{ mol} \cdot \text{dm}^{-3}$ NaCl. All data measured in our laboratory can therefore be represented by a single set of two equilibrium constants, *i.e.* a solubility and a deprotonation constant. It is noteworthy that in these experiments the uric acid solutions were supersaturated with respect to most of the corresponding hydrogenurate salts. If combination glass electrodes calibrated in standard buffer solutions are used for the *pH* measurement, uric acid solubilities can be calculated by the relation $[U]_{\text{tot}} = K_s(1 + K'_1/10^{-\text{pH}})$ where $\text{p}K'_1 = 5.33$ and $\text{p}K_s = 3.50$ or 3.21 for anhydrous uric acid or uric acid dihydrate, respectively.

Keywords. Uric acid; Solubility; Computer simulations; Renal calculi.

Löslichkeit von Harnsäure in Salzlösungen und künstlichem Urin

Zusammenfassung. Die Löslichkeit von wasserfreier Harnsäure wurde bei 37.0°C in verschiedenen Salzlösungen (Ionenstärke: $0.300 \text{ mol} \cdot \text{dm}^{-3}$), die gelegentlich organische Verbindungen enthielten, sowie in sogenanntem *Standard Reference Artificial Urine* gemessen. Die Ergebnisse wichen nicht signifikant von den Löslichkeiten ab, die zuvor in $0.150 \text{ mol} \cdot \text{dm}^{-3}$ NaCl erhalten wurden. Alle Daten, die in unserem Laboratorium ermittelt wurden, können demnach mit einem einzigen Satz von Gleichgewichtskonstanten beschrieben werden, d.h. mit je einer Löslichkeits- und Deprotonierungskonstante. Es ist bemerkenswert, daß in diesen Experimenten die Harnsäurelösungen in bezug auf die meisten der entsprechenden Hydrogenurate übersättigt waren. Wenn Einstabmeßketten, die in Standard-Pufferlösungen kalibriert wurden, zur *pH*-Messung verwendet werden, können die Harnsäurelöslichkeiten über die Beziehung $[U]_{\text{tot}} = K_s(1 + K'_1/10^{-\text{pH}})$ berechnet werden, wobei $\text{p}K'_1 = 5.33$ und $\text{p}K_s = 3.50$ (wasserfreie Harnsäure) oder 3.21 (Harnsäuredihydrat).

Introduction

Uric acid lithiasis is a widespread disease, possessing interesting epidemiological and characteristic clinical features [1]. The prevention and treatment of uric acid lithiasis are based upon the understanding of the physico-chemical principles underlying uric acid stone formation; amongst these, the dependencies of solubility

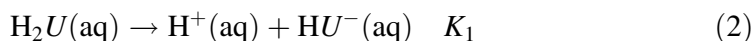
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on solution composition and pH are most important. In this work, a thorough investigation was conducted to assess the role of electrolytes on uric acid solubility under conditions most pertinent to urolithiasis. The media studied include standard reference artificial urine (abbreviated as SRAU [2]) and various salt solutions (occasionally containing organic compounds) of $0.300 \text{ mol} \cdot \text{dm}^{-3}$ ionic strength (which is believed to be of about the same ionic strength as that of urine). The dependence of solubility on salt concentration was also studied, and all experimental solubilities were then correlated using chemical equilibrium models. Furthermore, the effect of different salt bridge solutions and the use of combination electrodes were also studied and are discussed. As a result, a reliable method for the routine determination of uric acid saturation was derived.

Results and Discussion

Solubility of uric acid in various salt solutions and in SRAU

In the range $2 < -\log[\text{H}^+] < 9$, the concentration of urate, U^{2-} , is negligible, and so the solubility of uric acid can be described by considering two equilibria:



In our previous work [3], we obtained the solubility constant, K_s , and the first dissociation constant, K_1 , of uric acid by a least-squares analysis of solubility data measured at $0.150 \text{ mol} \cdot \text{dm}^{-3}$ NaCl in the whole $-\log[\text{H}^+]$ range under different starting conditions. The experimental quantity $[\text{U}]_{\text{tot}}$ equals the sum of $[\text{H}_2\text{U}]$ and $[\text{HU}^-]$ which can be rearranged to yield the equation

$$[\text{U}]_{\text{tot}} = [\text{H}_2\text{U}] + [\text{HU}^-] = K_s(1 + K_1/[\text{H}^+]) \quad (3)$$

For 37.0°C , the results were $pK_s = 3.50 \pm 0.01$ and $pK_1 = 5.19 \pm 0.04$.

In the present work, solubility measurements at 37.0°C in salt solutions containing 1.0 to $10.0 \text{ mmol} \cdot \text{dm}^{-3} \text{H}^+$, thus covering a $-\log[\text{H}^+]$ range where undissociated uric acid predominates in solutions, give similar results as the previous. In 0.050 to $0.500 \text{ mol} \cdot \text{dm}^{-3}$ (Na)Cl solutions, *i.e.* $[\text{Cl}^-]$ is kept constant at the stated value while $[\text{Na}^+]$ is not, no dependence of the solubility constant on the ionic strength could be detected, *i.e.* pK_s was found to be 3.50 ± 0.01 . The same result was obtained for $0.300 \text{ mol} \cdot \text{dm}^{-3}$ (K)Cl, $0.300 \text{ mol} \cdot \text{dm}^{-3}$ (Na)Cl + $0.050 \text{ mol} \cdot \text{dm}^{-3}$ creatinine, and for oxalate-free SRAU (see Experimental). It should be noted that the solubility of uric acid in artificial urine solutions can only be measured up to $-\log[\text{H}^+] \approx 5.5$; otherwise, calcium phosphates will precipitate. Although not very significant, the result was lower ($pK_s = 3.45 \pm 0.01$) for $0.100 \text{ mol} \cdot \text{dm}^{-3}$ $(\text{Na}_2)\text{SO}_4$, $0.100 \text{ mol} \cdot \text{dm}^{-3}$ $(\text{K}_2)\text{SO}_4$, and $0.300 \text{ mol} \cdot \text{dm}^{-3}$ (Na)Cl + $0.300 \text{ mol} \cdot \text{dm}^{-3}$ urea. Only for $0.100 \text{ mol} \cdot \text{dm}^{-3}$ (Ca)Cl₂, pK_s was slightly higher (3.51 ± 0.01).

At $-\log[\text{H}^+] > 3$, the solubility of uric acid in various salt and artificial urine solutions can be very well modelled by only two equilibrium constants determined previously for $0.150 \text{ mol} \cdot \text{dm}^{-3}$ NaCl [3], *i.e.* $pK_s = 3.50 \pm 0.01$ and $pK_1 = 5.19 \pm 0.04$ at 37.0°C . It should be noted that only a very small difference in the activity

coefficients y_1 of 1-1 electrolytes is found for $I = 0.150$ and $0.300 \text{ mol} \cdot \text{dm}^{-3}$ if the *Davies* equation is employed to estimate y_1 . Thus it seems reasonable to use the same pK_1 in this ionic strength range.

Therefore it can be concluded that the solubility of uric acid is independent of the ionic strength from 0.150 to $0.300 \text{ mol} \cdot \text{dm}^{-3}$ and from the background electrolyte of the solutions. This conclusion is supported by the work of *Sperling* and *de Vries* [4] who did not find any significant difference in the solubility of uric acid determined in buffer solutions containing $0.30 \text{ mol} \cdot \text{dm}^{-3}$ urea and real in urine. It should be noted that, although all our measurements were performed with anhydrous uric acid, analogous results are expected for the solubility of uric acid dihydrate. This means that the concentration and nature of the electrolytes should not have any significant effect on the solubility of uric acid dihydrate.

Our current results and those of *Sperling* and *de Vries* [4] are presented in Figs. 1-3. The experimental values measured at 37.0°C (symbols) agree very well with the calculated solubility curves (solid lines). The uric acid solubility curves for 25 , 32 , and 42°C (dotted lines), calculated using the equilibrium constants determined in our previous work [3], are also included for comparison. It is noteworthy that uric acid solutions were supersaturated with regard to most of the corresponding hydrogenurate salts. The solubility curves of these salts for 37°C in the same background electrolytes as that of uric acid solutions, calculated using previously published solubility products [3, 5, 6], are shown as dashed lines in Figs. 1-3.

The solubility curves of uric acid dihydrate for various temperatures are also shown in Fig. 3 as solid line for 37°C and dotted lines for other temperatures; these were calculated from the constants reported in Ref. [3]. It was found that

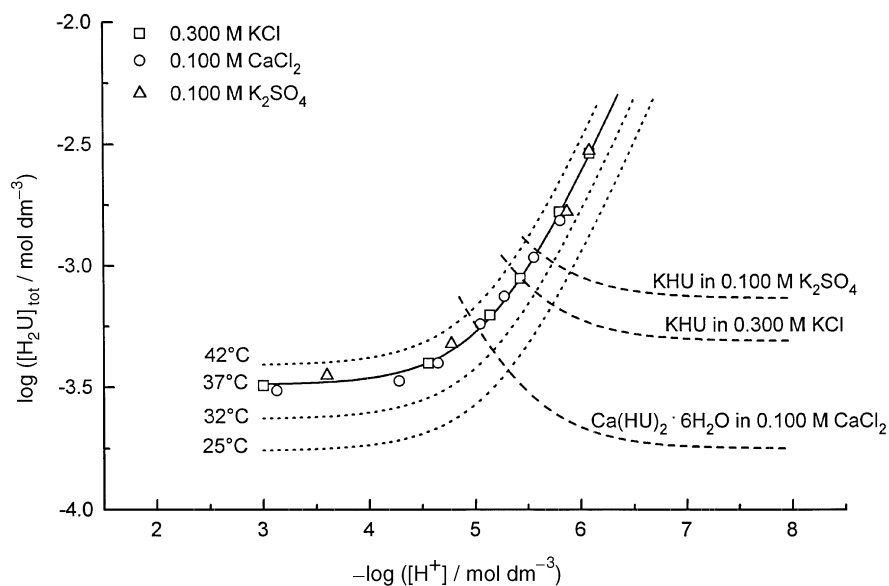


Fig. 1. Solubility of anhydrous uric acid in $0.300 \text{ mol} \cdot \text{dm}^{-3}$ KCl, $0.100 \text{ mol} \cdot \text{dm}^{-3}$ CaCl₂, and $0.100 \text{ mol} \cdot \text{dm}^{-3}$ K₂SO₄; experimental values measured at 37.0°C (symbols), calculated solubility curves of anhydrous uric acid at 37°C (solid line), calculated solubility curves of anhydrous uric acid at 25 , 32 , 42°C (dotted lines), calculated solubility curves of hydrogenurates at 37°C (dashed lines)

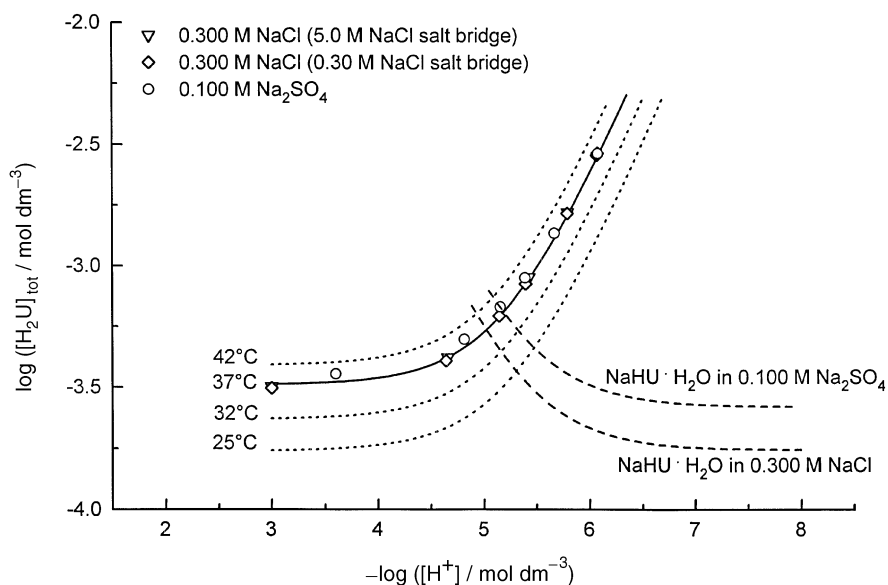


Fig. 2. Solubility of anhydrous uric acid in $0.100 \text{ mol} \cdot \text{dm}^{-3} \text{ Na}_2\text{SO}_4$, $0.300 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl}$ using a $5.0 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl}$ salt bridge, and $0.300 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl}$ using a $0.30 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl}$ salt bridge; experimental values measured at 37.0°C (symbols), calculated solubility curves of anhydrous uric acid at 37°C (solid line), calculated solubility curves of anhydrous uric acid at 25 , 32 , 42°C (dotted lines), calculated solubility curves of hydrogenurates at 37°C (dashed lines)

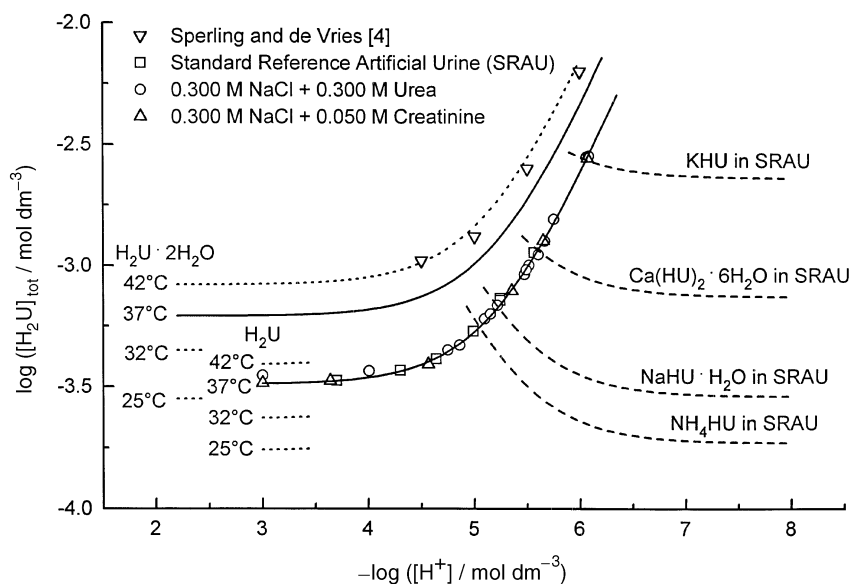


Fig. 3. Solubility of uric acid in standard reference artificial urine (SRAU), $0.300 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl} + 0.300 \text{ mol} \cdot \text{dm}^{-3} \text{ urea}$, and $0.300 \text{ mol} \cdot \text{dm}^{-3} \text{ NaCl} + 0.050 \text{ mol} \cdot \text{dm}^{-3} \text{ creatinine}$; Literature values [4] and experimental values measured in this study at 37.0°C (symbols), calculated solubility curves of anhydrous uric acid and uric acid dihydrate at 37°C (solid lines), calculated solubility curves of anhydrous uric acid and uric acid dihydrate at 25 , 32 , 42°C (dotted lines), calculated solubility curves of hydrogenurates at 37°C (dashed lines)

solubilities reported by *Sperling* and *de Vries* [4] agree much better with our data for the dihydrate rather than for anhydrous uric acid. In fact, the uric acid samples used by these authors were precipitated by acidification of real urine but were not characterized. Recently it was reported that under these conditions uric acid dihydrate is formed [7]. Thus it seems most likely that *Sperling* and *de Vries*' solubility data actually correspond to uric acid dihydrate.

Effect of different salt bridge solutions and use of combination electrodes for routine measurements of uric acid solubility

In most of the solubility measurements, a salt bridge solution of $5.0 \text{ mol} \cdot \text{dm}^{-3}$ NaCl was used so that the liquid junction potential E_j could be neglected [8]. Additionally, some experiments were performed with $0.30 \text{ mol} \cdot \text{dm}^{-3}$ NaCl salt bridge solution. In the latter case, the liquid junction potential must be considered in the $-\log[\text{H}^+]$ range at calibration, and it was calculated using the relation $E_j/\text{mV} = -51.2[\text{H}^+]/I_c$ which can be derived from a simplified form of the *Henderson* equation [8]. After the correction for liquid junction potential E_j , the results obtained with $0.30 \text{ mol} \cdot \text{dm}^{-3}$ NaCl salt bridge solution are very similar to those obtained with $5.0 \text{ mol} \cdot \text{dm}^{-3}$ NaCl salt bridge solution without liquid junction potential correction (Fig. 2). This finding confirms the advantage of using a concentrated salt bridge solution.

Following the calibration in standard buffer solutions, commercial combination glass electrodes were used to measure the *pH* of the calibrating solutions S_A (see Experimental), at 37.0°C . The results obtained (2.15 ± 0.04 ; for sulfate solutions a correction of 0.60 was applied, see Experimental) agreed well with the expected values (2.14 ± 0.02) calculated from the relationship $\text{pH} = -\log([\text{H}^+]_{y_1}) \pm 0.02$ [9] where y_1 is calculated using *e.g.* the *Davies* equation. The small standard deviation found in this work indicates only slight differences in the activity coefficients of H^+ in various salt solutions of the same ionic strength. Therefore, for routine determination of uric acid saturation, combination electrodes which have been calibrated with standard buffer solutions can be employed to measure the *pH* of the solutions. In this case, the experimentally determined uric acid concentration can be compared to the uric acid solubility calculated by the relation $[U]_{\text{tot}} = K_s(1 + K'_1/10^{-\text{pH}})$ with a modified $\text{p}K'_1 = \text{p}K_1 - \log y_1 = 5.33$ and $\text{p}K_s = 3.50$ or 3.21 for anhydrous uric acid or uric acid dihydrate, respectively. If the measured uric acid concentration exceeds the calculated value, the solution (*e.g.* urine) is supersaturated; otherwise, it is undersaturated.

Conclusion

In this work, the solubility of uric acid was thoroughly investigated at 37.0°C . Excellent agreement was obtained between the experimentally determined solubilities and the calculated values in all media studied. The results show that concentration and nature of the electrolytes have very negligible effect on the solubility of uric acid. Consequently, the solubility of uric acid in all solutions can be modelled by the relation $[U]_{\text{tot}} = K_s(1 + K_1/[\text{H}^+])$ with only two equilibrium constants ($\text{p}K_s = 3.50 \pm 0.01$ and $\text{p}K_1 = 5.19 \pm 0.04$).

For routine determination of uric acid saturation, after being calibrated with standard buffer solutions, commercial combination glass electrodes can be employed to measure the pH of the solutions. The uric acid solubility can then be calculated using $[U]_{\text{tot}} = K_s(1 + K'_1/10^{-pH})$ with $pK'_1 = 5.33$ and $pK_s = 3.50$ or 3.21 for anhydrous uric acid or uric acid dihydrate, respectively.

Experimental

Analytical reagent grade chemicals, concentrated volumetric standards of HCl and NaOH, A-grade glassware, and bidistilled water were employed throughout. High-purity uric acid ($C_5H_4N_4O_3$, 99%+, Aldrich-Chemical Co.) was used directly. X-Ray diffraction analysis and scanning electron microscopy confirmed that this commercial sample was pure anhydrous uric acid.

All solubility measurements were performed at $37.00 \pm 0.02^\circ\text{C}$ in a similar manner to that reported previously (*e.g.* Ref. [3]). In the solubility cell [10, 11], the initial solution was percolated by a prehumidified N_2 -gas stream through the solid phase of uric acid. In the pH variation method [12] employed here, the ionic strength was kept constant, and $[OH^-]_{\text{ini}}$ was varied systematically from one experiment to another. An ORION 940 pH meter was used to record the pH values of the solutions. In each experiment, a constant pH value was obtained after *ca.* 6 h indicating (metastable) solubility equilibrium. The following salt solutions of $I_c = 0.300 \text{ mol} \cdot \text{dm}^{-3}$ were used: $0.300 \text{ mol} \cdot \text{dm}^{-3}$ NaCl, $0.300 \text{ mol} \cdot \text{dm}^{-3}$ KCl, $0.100 \text{ mol} \cdot \text{dm}^{-3}$ Na_2SO_4 , $0.100 \text{ mol} \cdot \text{dm}^{-3}$ K_2SO_4 , $0.100 \text{ mol} \cdot \text{dm}^{-3}$ $CaCl_2$, $0.300 \text{ mol} \cdot \text{dm}^{-3}$ NaCl + $0.300 \text{ mol} \cdot \text{dm}^{-3}$ urea, and $0.300 \text{ mol} \cdot \text{dm}^{-3}$ NaCl + $0.050 \text{ mol} \cdot \text{dm}^{-3}$ creatinine. The following composition of SRAU was used: $0.1055 \text{ mol} \cdot \text{dm}^{-3}$ NaCl, $0.0323 \text{ mol} \cdot \text{dm}^{-3}$ NaH_2PO_4 , $0.00321 \text{ mol} \cdot \text{dm}^{-3}$ $Na_3C_6H_5O_7$ (sodium citrate), $0.00385 \text{ mol} \cdot \text{dm}^{-3}$ $MgSO_4$, $0.01695 \text{ mol} \cdot \text{dm}^{-3}$ Na_2SO_4 , $0.0637 \text{ mol} \cdot \text{dm}^{-3}$ KCl, $0.00575 \text{ mol} \cdot \text{dm}^{-3}$ $CaCl_2$, and $0.0455 \text{ mol} \cdot \text{dm}^{-3}$ NH_4^+ . Oxalate was omitted from this solution to avoid the precipitation of Ca oxalate. The solubility constant of undissociated uric acid was also measured in $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ HCl/NaCl solutions of ionic strengths of 0.050, 0.100, 0.150, 0.300, and $0.500 \text{ mol} \cdot \text{dm}^{-3}$.

Generally, the galvanic cells employed for pH measurements can be represented as $Ag|AgCl|5.0$ or $0.30 \text{ mol} \cdot \text{dm}^{-3}$ NaCl|5.0 or $0.30 \text{ mol} \cdot \text{dm}^{-3}$ NaCl| S_A or S|glass electrode (A) where S_A is the calibrating solution and S is the test solution. Schott H2680 glass electrodes and home-made silver-silver chloride reference electrodes with *Wilhelm* type salt bridges [13] were used to measure $-\log[H^+]$ of the solution during the reaction with the solid phase. Cell A was calibrated before and after each experiment with the respective salt solutions S_A which contained $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ H^+ from the corresponding acid. In the case of the sulfates, the second dissociation step of sulfuric acid must be taken into account. A pK_2 value of 1.50 [14], estimated for the prevailing conditions, resulted in a $-\log[H^+]$ of 2.60 instead of 2.00 for the calibrating solution. For the solubility measurements in SRAU, a $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ HCl/ $0.290 \text{ mol} \cdot \text{dm}^{-3}$ KCl solution was used for calibration because this solution is believed to have about the same ionic strength and hence the same activity coefficient of H^+ as SRAU. It is noteworthy that $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ HCl/ $0.290 \text{ mol} \cdot \text{dm}^{-3}$ NaCl + $0.300 \text{ mol} \cdot \text{dm}^{-3}$ urea and $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ HCl/ $0.290 \text{ mol} \cdot \text{dm}^{-3}$ NaCl + $0.050 \text{ mol} \cdot \text{dm}^{-3}$ creatinine solutions cannot be used for calibration because the protonation of urea and creatinine leads to a higher initial pH value of these solutions. Therefore, the electrodes were calibrated in $10.0 \text{ mmol} \cdot \text{dm}^{-3}$ HCl/ $0.290 \text{ mol} \cdot \text{dm}^{-3}$ NaCl solution. It should also be noted that, under the present experimental conditions, the decomposition of urea to ammonium hydrogencarbonate was observed by a continuous but very slow increase of pH (which did not stop even after several days), whereas solid uric acid reaches equilibrium with aqueous solutions within hours. Accordingly, the effect of the decomposition of urea is insignificant in the present study of uric acid solubility in urea solutions. A salt bridge solution of $5.0 \text{ mol} \cdot \text{dm}^{-3}$ NaCl was used in most of the solubility measurements, and a $0.30 \text{ mol} \cdot \text{dm}^{-3}$ NaCl salt bridge solution was employed in some experiments. Also, the pH of the calibrating H^+ /salt solutions was sometimes measured with commercial

combination glass electrodes (Orion Ross electrodes) which were calibrated in three standard buffer solutions (Merck, $pH=4.00\pm 0.02$, 7.00 ± 0.02 , and 9.00 ± 0.02). The pH values of the standard buffer solutions were corrected to 37.0°C , the correction applied (given by Merck) was -0.13 , -0.03 , and 0.01 for pH 4.00, 7.00, and 9.00, respectively.

At the end of a solubility measurement, the saturated uric acid solution was diluted with $0.100\text{ mol}\cdot\text{dm}^{-3}$ NaOH solution, and the total concentration of urate was determined at 293.6 nm using a Perkin Elmer Lambda 15 UV/Vis Spectrometer with $0.100\text{ mol}\cdot\text{dm}^{-3}$ NaOH as reference solution. It was found that at this wavelength the pH value of the solutions does not have strong influence on the peak position and absorption of urate. *Beer-Lambert's* law is well obeyed in the concentration range from 0.0250 to $0.1500\text{ mmol}\cdot\text{dm}^{-3}$. A linear calibration curve was obtained with a regression coefficient of 0.99995 , and the molar absorption coefficient was $13375\text{ dm}^3\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. For uric acid solutions containing Ca^{2+} and/or Mg^{2+} , filtration was needed to remove the hydroxide precipitates after $0.100\text{ mol}\cdot\text{dm}^{-3}$ NaOH was added to the solutions. It is important to note that urate solutions are not very stable with time (in three days the absorption of a solution decreases to two thirds of the initial value). The spectrophotometric measurements were therefore carried out immediately after the preparation of urate solutions.

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