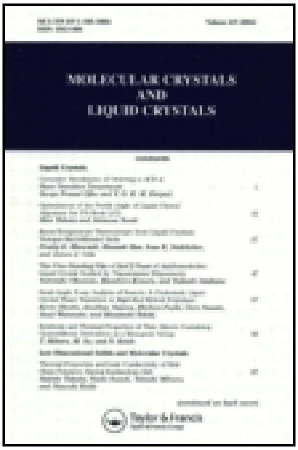


Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Uric Acid Dye Inclusion Crystals

Ryan E. Sours [a], Dorothy A. Fink [a], Kristin A. Cox [a] & Jennifer A. Swift [a]

[a] Department of Chemistry, Georgetown University, Washington, DC

Version of record first published: 31 Aug 2006

To cite this article: Ryan E. Sours, Dorothy A. Fink, Kristin A. Cox & Jennifer A. Swift (2005): Uric Acid Dye Inclusion Crystals, Molecular Crystals and Liquid Crystals, 440:1, 187-193

To link to this article: http://dx.doi.org/10.1080/15421400590958250

Mol. Cryst. Liq. Cryst., Vol. 440, pp. 187–193, 2005
Copyright © Taylor & Francis Inc.
ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400590958250

Taylor & Francis
Taylor & Francis Group

Uric Acid Dye Inclusion Crystals

Ryan E. Sours
Dorothy A. Fink
Kristin A. Cox
Jennifer A. Swift
Department of Chemistry, Georgetown University, Washington, DC

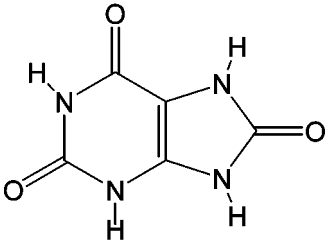
*Crystallization in the presence of molecular dye probes has been used as a means of discerning surface recognition events that occur during the growth of uric acid (**UA**) and uric acid dihydrate (**UAD**), which are known components of some human kidney stones. The growth of synthetic **UA** and **UAD** crystals from supersaturated aqueous solutions containing seven different cationic dyes (Thionin, New Methylene Blue, Neutral Red, Acridine Yellow G, Safranine O, Bismarck Brown Y, and Bismarck Brown R) yielded dye-included crystals in all cases. Herein we describe the specific inclusion patterns and/or habit modification resulting from these growth conditions.*

Keywords: cationic dyes; crystal habits; inclusion compounds; uric acid; uric acid dihydrate

INTRODUCTION

Uric acid is a waste product of protein metabolism that is removed from the body via the kidneys. Many factors (e.g., low urine pH, dehydration, chemotherapy, etc.) can result in increased concentrations of uric acid and its subsequent crystallization *in vivo*. The pH of fluid passing through the kidneys ranges from 4.8–7.8 [1]. The solubility of uric acid is pH dependent, and a reduction in pH lowers its solubility [2]. Urinary solutions under these naturally low pH conditions, or pure supersaturated uric acid solutions on the laboratory bench, can yield crystals of either neutral uric acid (**UA**) or uric acid dihydrate (**UAD**).

Address correspondence to Jennifer A. Swift, Department of Chemistry, Georgetown University, Washington, DC 20057-1227. E-mail: jas2@georgetown.edu



Uric Acid

The crystal structures of both **UA** and **UAD** are known. **UA** adopts a layered motif, (space group $P2_1/a$ and unit cell dimensions $a = 14.464(3)$, $b = 7.403(2)$, $c = 6.208(1)$ Å, and $\beta = 65.10(5)^\circ$) [3] in which each layer consists of parallel ribbons of molecules hydrogen-bonded head-to-head and tail-to-tail, with the ribbon plane perpendicular to the *bc* plane. Ribbons in adjacent layers are also hydrogen-bonded together and offset by ~62°. **UAD** single crystals (space group $P2_1/c$ with $a = 7.237(3)$, $b = 6.363(4)$, $c = 17.449(11)$, and $\beta = 90.51(1)^\circ$) [4] also show a clear lamellar packing. Ribbons found within **UAD** layers are topologically similar to those seen in **UA**, however hydrogen-bonding interactions between layers are mediated by water molecules. Most **UAD** crystals also exhibit a significant level of disorder and/or twinning.

Synthetic **UA** and **UAD** crystals grown under pure laboratory conditions exhibit significantly different habits and colors to uric acid crystals grown *in vivo*. **UA** crystals grown from pure supersaturated aqueous solutions deposit as transparent rectangular plates, with large (100) faces bounded by (210), (201), (001), and sometimes very small (121) faces [5]. Synthetic **UAD** deposits as clear rectangular plates, with large (001) faces bounded by (011) and (102) and infrequently (210) [5]. Uric acid crystals extracted from human kidney stones are irregularly shaped and colored, indicating the inclusion of impurities (Fig. 1). Interactions between the naturally derived impurities and growing uric acid crystal surfaces, may serve to alter the relative growth rates and lead to the unusual morphologies observed. Some of the occluded impurities have previously been identified [6–9].

Investigations into the crystallization of uric acid in the presence of impurities began as early as the 1930s, when Gaubert [10] first examined its growth from solutions containing assorted synthetic and natural dyes. Kleeberg also performed a series of dye experiments on uric acid in the 1970s [9,11]. Though these early studies offered little insight into a rational molecular-level understanding of the recognition events between the dyes and crystal surfaces, they did show that it was

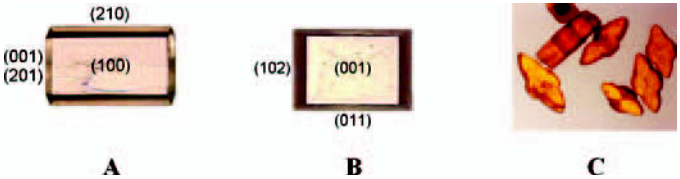


FIGURE 1 Micrographs of uric acid crystals. (A) Synthetic crystal of **UA** grown from supersaturated aqueous solution at 37°C, (B) Synthetic crystal of **UAD** grown from supersaturated aqueous solution at 24°C, (C) Natural uric acid dihydrate crystals (provided courtesy of Louis C. Herring and Company).

possible to include dye into these single crystal matrixes. More recent work by Kahr [12] on different crystal hosts has demonstrated that dye probes can be used as a very effective tool for discerning surface-recognition events during crystal growth.

Upon revisiting some of Gaubert's and Kleeberg's dyes, studies in our laboratory found that dye recognition can be quite specific in some cases. For example, Methylene Blue (**MB**) was found to selectively include in {001} and {201} growth sectors of **UA**, but to include nonspecifically in **UAD** crystal matrixes [13]. Only a fraction of the dye molecules in solution were actually included in the uric acid matrixes (~1 dye per 10^4 uric acid), and **UA-MB** and **UAD-MB** crystals grown under a broad range of dye concentrations exhibited rectangular habits identical to pure synthetic uric acid crystals. Microspectrophotometry was used to determine the orientation of the dye in **UA-MB** crystals. This technique is described in greater detail in Reference 13.

In contrast, our studies with Acriflavine Neutral (**AN**) revealed slightly different inclusion phenomena [14]. Although **AN** was selectively included and oriented in both **UA** and **UAD** matrixes, increasing solution concentrations of dye were found to alter the morphology of **UA** such that (121) faces became more pronounced while (001) and (201) faces grow out of existence. However, **UAD** hosts showed no habit modification, even when grown in the presence of the highest **AN** solution concentrations. The disparity in dye inclusion and habit changes in the presence of **MB** and **AN** is clearly due to subtle differences in the structure of the two dyes and their interactions with growing uric acid crystal surfaces.

In order to fine tune our understanding of how dye-surface chemical interactions result in the incorporation of dye and/or habit modification of uric acid crystals, we have adopted a combinatorial approach by growing **UA** and **UAD** crystals in the presence of a broader variety of other dyes. Some of the dyes were chosen for their structural similarity to methylene blue or acriflavine neutral, while others exhibit a larger degree of conformational flexiblity. The current work summarizes the results for growth of **UA** and **UAD** in the presence of seven new dyes:Thionin (**Th**), New Methylene Blue (**NMB**), Neutral Red (**NR**), Acridine Yellow G (**AYG**), Safranine O (**SO**), Bismarck Brown Y (**BBY**), and Bismarck Brown R (**BBR**).

METHODS

Pure uric acid crystals were grown by dissolving ~38 mg of uric acid (Aldrich, 99 + %) in 200 mL of boiling water. The resulting uric acid solutions ($\sim 1 \times 10^{-3}$ M) are then distributed into vials, half of which were left at room temperature (24°C) and half submerged in a 37°C water bath for several days. **UA** crystals grew at 37°C while **UAD** growth is favored at room temperature.

In order to grow uric acid crystals with dye inclusions, the dye must be added before crystallization occurs. Crystals were grown from solutions with dye concentrations ranging from 1×10^{-6} M to 3×10^{-4} M. The dyes investigated were Thionin [#78338-22-4], New Methylene Blue [#6586-05-6], Neutral Red [#553-24-2], Acridine Yellow G [#135-49-9], Safranine O [#477-73-6], Bismarck Brown Y [#10114-58-6], and Bismarck Brown R [#5421-66-9]. All dyes were of the highest purity available from Aldrich and used without further purification.

RESULTS AND DISCUSSION

The results described below are also summarized graphically in Figure 2. While the dye inclusion pattern varied among the dyes and crystal forms, the included dye molecules were invariably oriented as evidenced by their linear dichroism observed under polarized light microscopy.

New Methylene Blue (**NMB**) was chosen for its structural similarity to methylene blue (**MB**). Despite the additional methyl groups at each end of the molecule and the longer ethyl sidechain on both amines, the inclusion of **NMB** was indistinguishable from **MB**. Although dye was included in both **UA** and **UAD**, only UA showed a specific hourglass inclusion pattern indicating recognition through {001} and {201} growth sectors. No significant habit change was observed for either crystal form, though tiny {121} faces in **UA-NMB** were sometimes observed.

Thionin (Th) was also chosen for its structural similarity to **MB**. We had hoped that the primary amine functionalities would facilitate hydrogen bonding with the growing uric acid crystal surface. Neither specific inclusion or habit changes were observed for **UAD-Th**.

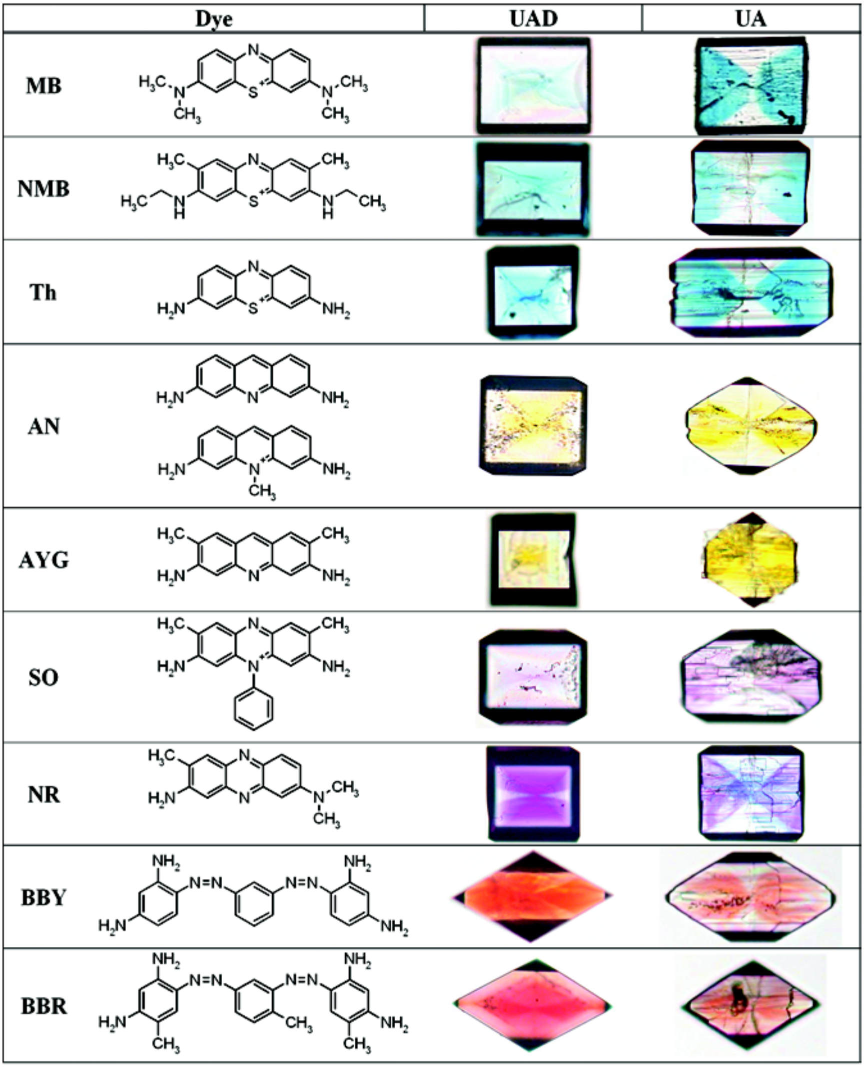


FIGURE 2 The molecular structures of the dyes investigated, with pictures of their corresponding **UAD** and **UA** inclusion crystals.

However, thionin inclusions in **UA-Th** matrixes were highly specific, and dye inclusion resulted in the appearance of {121} faces.

Acridine Yellow G (**AYG**) is structurally similar to acriflavine neutral (**AN**), with the addition of methyl groups at the 2- and 7- positions of the molecule. While our previous studies with **AN** showed a habit change for only **UA** and specific inclusion in both **UA** and **UAD** hosts, AYG was included specifically and effected habit changes in **UA** as the solution dye concentration increased, but was not selectively included in **UAD** crystals.

Safranine O (SO) was used to probe possible steric effects, as the addition of a phenyl ring to the central nitrogen makes **SO** substantially larger than **AYG**. We hypothesized that the increased bulk of **SO** might preclude its inclusion in uric acid crystals. However, we observed selective inclusion in **UA**, with habit modification of both **UA** and **UAD**. We hypothesize that perhaps the rotational flexibility of the phenyl ring allows **AYG** to fit inside the crystal matrix despite its size.

Neutral Red (**NR**) was one of the early dyes reported by Gaubert [10] to be included in uric acid crystals. Surprisingly, **NR** is selectively included in both **UA** and **UAD** and it also modifies the habit of **UA**. The selective inclusion of **NR** in **UAD** is difficult to rationalize based on the dye's similarity to both **AYG** and **MB**.

Gaubert [10] also reported that Bismarck Brown dye was incorporated in uric acid crystals, although he did not specify which variety of the dye he used. We found **Bismarck Brown Y** (**BBY**) to selectively include in both **UA** and **UAD**, and to substantially modify the crystal habits of both. The inclusion of **Bismarck Brown R** (**BBR**) is identical to that of **BBY**, despite the addition of three methyl groups on the aromatic rings. Moreover, the solution dye concentration needed for **BBY** and **BBR** inclusion and habit change is lower than that for any of the other dyes investigated. The conformational flexibility of the dye likely allows it to fit within the uric acid crystal matrix while the four primary amines are available for hydrogen bonding.

CONCLUSIONS

Our study of dye inclusion in uric acid crystal matrixes is ongoing. While predicting the ability of a dye to selectively include in **UA** or **UAD** based on structure alone may not be possible, we have observed the trend that the presence of primary amine functionalities tends to favor selective inclusion. Also, the presence of primary amines (over secondary or tertiary amines) as well as steric effects may drive crystal habit modification by included dyes.

REFERENCES

[1] Altman, P. L. & Dittmer, D. S. (1971). *Blood and Other Body Fluids*, Federation of American Societies for Experimental Biology: Bethesda, MD.
[2] Konigsberger, E. & Wang, Z. (1999). *Monatshefte fur Chemie*, *130*, 1067.

[3] Ringertz, H. (1966). *Acta. Cryst.*, *20*, 397.

[4] Parkin, S. & Hope, H. (1998). *Acta. Cryst. B*, *54*, 339.

[5] Rinaudo, C. & Boistelle, R. (1980). *J. Cryst. Grow.*, *49*, 569.

[6] Iwata, H., Kamei, O., Abe, Y., Nishio, S., Wakatsuki, A., Ochi, K., & Takeuchi, M. (1988). *Journal of Urology*, *139*, 607.

[7] Grases, F., Ramis, M., Villacampa, A. I., & Costa-Bauza, A. (1999). *Urologia Internationalis*, *62*, 201.

[8] Pinto, B., Rocha, E., & Ruiz-Marcellan, F. J. (1976). *Kidney International*, *10*, 437.

[9] Kleeberg, J., Warski, E., & Shalitin, J. (1974). In: *Advances in Experimental Medicine and Biology*, Sperling, O., DeVries, A., & Wyngaarden, J. B. (Eds.), Plenum Press: New York, Vol. V41B, 821.

[10] Gaubert, P. (1936). *Comptes Rendus*, *202*, 1192.

[11] Kleeberg, J. (1976). *Israel J. Med. Sci.*, *12*, 73.

[12] Kahr, B. & Gurney, R. W. (2001). *Chem. Rev.*, *101*, 893.

[13] Sours, R. E., Fink, D. A., & Swift, J. A. (2002). *J. Am. Chem. Soc.*, *124*, 8630.

[14] Fink, D. A., Sours, R. E., & Swift, J. (2003). *A. Chem. Mater.*, *15*, 2718.