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Calcium Coordination Solids for pH-Triggered Release of Olsalazine

Dana J. Levine, Miguel I. Gonzalez, Christina M. Legendre, Tomče Runčevski, Julia Oktawiec, Kristen A. Colwell, and Jeffrey R. Long*

Abstract: Calcium coordination solids were synthesized and evaluated for delivery of olsalazine (H_4olz), an anti-inflammatory compound used for treatment of ulcerative colitis. The materials include one-dimensional Ca(H_2olz)·4H_2O chains, two-dimensional Ca(H_2olz)·2H_2O sheets, and a three-dimensional metal–organic framework Ca(H_2olz)·2DMF (DMF = *N*,*N*-dimethylformamide). The framework undergoes structural changes in response to solvent, forming a dense Ca(H_2olz) phase when exposed to aqueous HCl. The compounds Ca(H_2olz)·*x*H_2O (*x* = 0, 2, 4) were each pressed into pellets and exposed to simulated gastrointestinal fluids to mimic the passage of a pill from the acidic stomach to the pH-neutral intestines. All three calcium materials exhibited a delayed release of olsalazine compared to Na_2(H_2olz), the commercial formulation, illustrating how formulation of a drug within an extended coordination solid can serve to tune its solubility and performance.

Olsalazine is a prodrug of the anti-inflammatory 5-aminosalicylic acid (5-ASA, Figure 1a), which is prescribed as the first line of treatment for patients with idiopathic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.[1] Since 5-ASA alone does not reach the colon when administered orally, an enteric coating is required for formulation.[2] Alternatively, 5-ASA is prepared as an azo-linked prodrug such as olsalazine, which is cleaved by bacterial azoreductases in the colon where the concentration of bacteria is highest (Figure 1b).[3] Patients with ulcerative colitis often require daily multigram doses of 5-ASA to achieve therapeutic concentrations in the colon,[4] so it is desirable to minimize the amount of excipients or adjuvant molecules in the dosage form. Among approved prodrugs of 5-ASA, olsalazine is the most efficient by weight, since it is a homodimer of two 5-ASA molecules, whereas sulfasalazine and balsalazide are heterodimers of 5-ASA and carrier molecules.

While olsalazine disodium is effective for treatment of active ulcerative colitis and for maintenance of remission,[5] as much as 35% of patients experience diarrhea as a side effect, causing over 10% of patients to discontinue treatment.[6] These dose-dependent effects are also observed with other azo-linked prodrugs of 5-ASA[7] and are attributed to increased secretion of anions and inhibition of NaCl absorption in the small intestine.[8] Such side effects may be diminished in formulations that minimize olsalazine release throughout the upper gastrointestinal tract. To this end, olsalazine has been incorporated into materials such as polymer matrices and hydrogels;[9] however, the high molecular weights of the additives required for preparation of these materials result in a low weight-percent of olsalazine, which may be problematic due to dosing limitations.

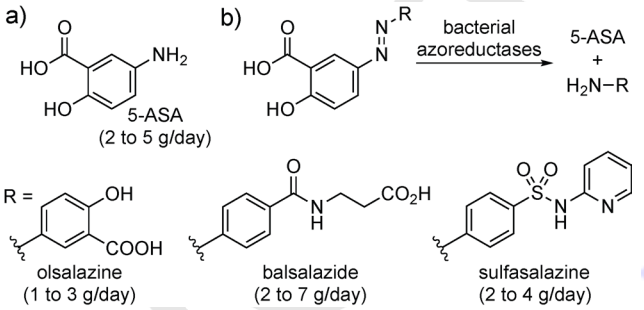

Figure 1. Structure and recommended oral dose[2] for (a) 5-ASA and (b) azo-linked prodrugs, which release 5-ASA and a carrier molecule. In olsalazine, the carrier is another equivalent of 5-ASA.

Metal–organic frameworks and coordination solids have been increasingly investigated for potential applications in drug delivery, owing to their structural diversity and high drug-loading capacities.[10] A variety of metal–organic materials have been made using bioactive linkers,[11] and such materials can serve as platforms for release of both bioactive molecules and metal ions.[12] While a handful of biocompatible olsalazine coordination solids are known,[13] only one has yet been tested for time-dependent drug release.[13b] We therefore sought to evaluate the drug-release properties of a wider array of biocompatible olsalazine coordination solids. In particular, we focused on calcium-based solids because Ca^{2+} is known to produce a variety of architectures with dicarboxylate ligands.[14] Additionally, the Ca^{2+} component itself may provide therapeutic benefits for patients with ulcerative colitis.[15] In this study, calcium coordination solids that form one-, two-, and three-dimensional structures with olsalazine (H_4olz) were synthesized, characterized, and investigated as potential alternatives to the existing olsalazine disodium formulation.

A one-dimensional coordination solid Ca(H_2olz)·4H_2O (**1**, Figure 2) has been reported previously, where the material was made by slow evaporation from a water-ethanol mixture.[13a] However, we have accessed this compound through a more rapid synthesis from $Ca(NO_3)_2$·4H_2O and olsalazine that requires only water as the solvent and a reaction time of hours instead of weeks. Its crystal structure consists of pentagonal bipyramidal Ca^{2+} ions that are each coordinated to four water molecules and three carboxylate oxygen atoms from two different olsalazine molecules, where one carboxylate coordinates in a monodentate

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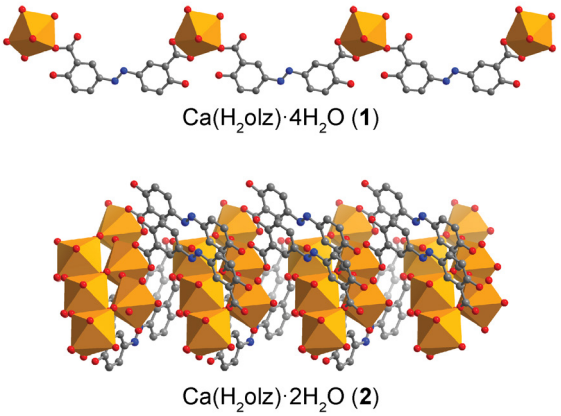

Figure 2. Portions of the crystal structures of $Ca(H_2olz)\cdot 4H_2O$ (**1**) containing one-dimensional chains[13a] and $Ca(H_2olz)\cdot 2H_2O$ (**2**) featuring two-dimensional sheets. Grey, blue, and red spheres represent carbon, nitrogen, and oxygen atoms, respectively; hydrogen atoms are omitted for clarity. Orange surfaces represent the polyhedra formed by the first coordination sphere of the Ca^{2+} ions.

fashion and the other in a bidentate fashion. Each olsalazine unit thus bridges two Ca^{2+} ions to produce one-dimensional chains.

We have also discovered a new two-dimensional phase, $Ca(H_2olz)\cdot 2H_2O$ (**2**, Figure 2), and determined its structure by single-crystal X-ray diffraction. This phase can be obtained through reaction conditions similar to those developed for the one-dimensional chains. While sonication or stirring of the reaction mixture at elevated temperature tends to favor the one-dimensional phase, leaving the reaction undisturbed tends to afford the two-dimensional phase. This behavior suggests a delicate balance in the reaction kinetics and thermodynamics that govern the formation of one phase over the other.[16] The sheets within this crystal structure are comprised of pentagonal bipyramidal Ca^{2+} ions, which are each coordinated to two water molecules in a *cis* geometry and five carboxylate oxygen atoms from the bridging olsalazine units.

Reaction of $Ca(NO_3)_2\cdot 4H_2O$ and olsalazine in a mixture of DMF and ethanol under solvothermal conditions yields single crystals of the three-dimensional metal–organic framework $Ca(H_2olz)\cdot 2DMF$ (**3**·DMF, Figure 3a). In this structure, the Ca^{2+} ions exhibit an octahedral coordination environment with two DMF molecules bound in the axial positions and carboxylate oxygen atoms from four different olsalazine ligands bound in the equatorial positions.

The framework exhibits remarkable flexibility, undergoing significant structural changes in the presence of different solvents. Similar behavior has been observed for other calcium frameworks with dicarboxylate ligands.[14b-e] Exposure of **3**·DMF to wet methanol produces a new phase, $Ca(H_2olz)\cdot 2MeOH\cdot H_2O$ (**3**·MeOH), with a structure that was determined from powder X-ray diffraction data (Figure 3b). Comparison of the structures revealed that the two coordinated DMF molecules in the original material are replaced by one methanol and one water molecule; an additional methanol molecule resides in the pore. Although the connectivity of the olsalazine ligand to the Ca^{2+} ion is maintained throughout this flexing, there is a dramatic shift in the positions of the solvent molecules. In the original structure, the DMF molecules are *trans* to one another, whereas the bound solvent molecules in the methanol structure are *cis* to one another (Figure 3, right).

Immersion of either **3**·DMF or **3**·MeOH in 100-mM aqueous HCl irreversibly generates a third phase, likely with an accompanying change in ligand coordination mode (Figure 4). While the powder pattern of this phase could not be indexed to determine the structure, thermogravimetric analysis showed a single mass loss event at ~250 °C corresponding to decomposition (Figure S10). In conjunction with elemental analysis, this result corroborates a dense phase with the formula $Ca(H_2olz)$ (**3**) in which no solvent is present.

To evaluate the potential utility of $Ca(H_2olz)\cdot xH_2O$ coordination solids in the treatment of ulcerative colitis (x = 4, 2, and 0 for **1**, **2**, and **3**, respectively), each material was tested for drug release in comparison with $Na_2(H_2olz)$, which is the salt used in the commercial formulation (available as Dipentum). Each material was pressed into a pellet and exposed to solutions that mimic the pH of the stomach, small intestine, and colon (Figure 5). The pH and composition of the release medium was changed by addition of buffers in accordance with the expected transit times of a pill through the gastrointestinal tract: the first two hours were held at pH 1.1, the next two hours at pH 6.0, and the final six hours at pH 7.3. The vessels containing the pellet in release media were shaken at 60 rpm and 37 °C to simulate the motion and temperature of the body.

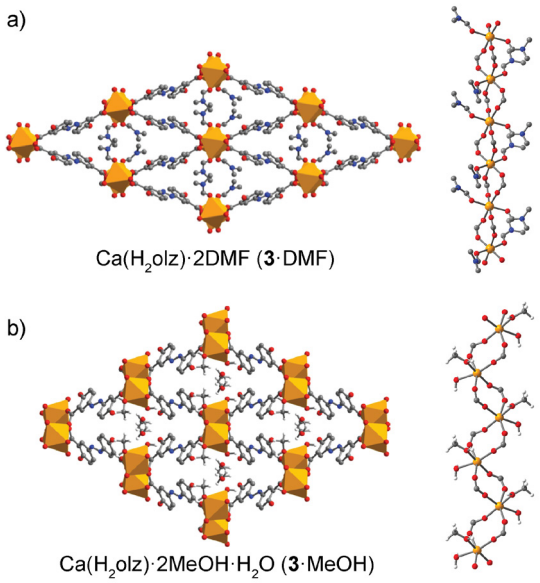

Figure 3. Portions of the crystal structures of (a) $Ca(H_2olz)\cdot 2DMF$ (**3**·DMF) and (b) $Ca(H_2olz)\cdot 2MeOH\cdot H_2O$ (**3**·MeOH) as viewed down *c*-axis (left) and the *b*-axis (right). Grey, blue, red, and white spheres represent C, N, O, and H atoms, respectively; some hydrogen atoms are omitted for clarity. Orange surfaces represent the polyhedra formed by the first coordination sphere of the Ca^{2+} ions.

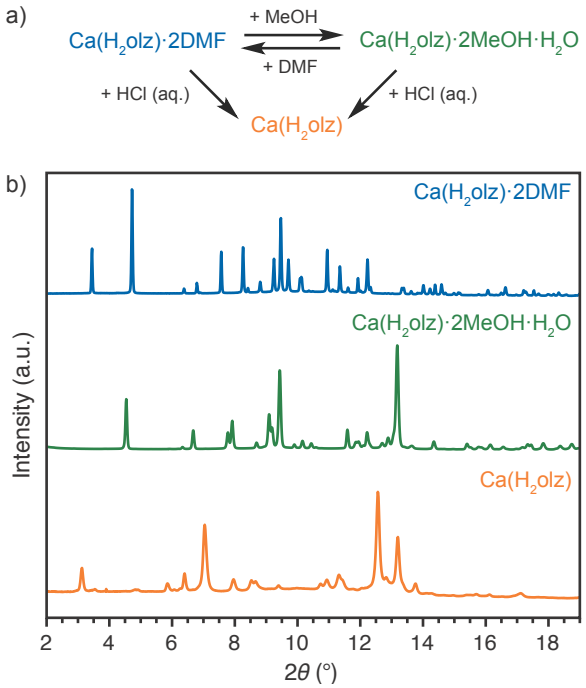

Figure 4. (a) Scheme illustrating the reversibility between the DMF and methanol-solvated structures of the three-dimensional $Ca(H_2olz)$ metal–organic framework and the irreversible change that occurs after exposure to 100-mM aqueous HCl (**3**). (b) Flexibility of the three-dimensional $Ca(H_2olz)$ structure was analyzed by powder X-ray diffraction (λ = 0.72768 Å).

Sample pellets were prepared from pure material without binders or other agents typically used for pill preparation in the pharmaceutical industry. This was done in order to probe the properties of each material without influence from any excipients. Due to this method of preparation, however, pellets were susceptible to disintegration, which can accelerate the observed dissolution rates. The calcium-olsalazine chains (**1**) and sheets (**2**), as well as the sodium-olsalazine material, partially disintegrated once in contact with the solution. The variability in pellet integrity likely contributed to the large observed standard deviation. Notably, the $Ca(H_2olz)$ framework (**3**) consistently resisted disintegration throughout the release experiments.

All three of the $Ca(H_2olz)$ materials outperformed $Na_2(H_2olz)$ by providing slower release of olsalazine in the simulated gastrointestinal environment. While all materials resisted dissolution at pH 1.1, the $Na_2(H_2olz)$ dissolved more rapidly than the $Ca(H_2olz)$ materials at pH 6.0 and above. The difference in dissolution rates is particularly clear when comparing the amount of drug released at the 4-h time point, where over 90% of the olsalazine had been released from $Na_2(H_2olz)$ while about 50% had been released from the $Ca(H_2olz)·4H_2O$ chains and the $Ca(H_2olz)·2H_2O$ sheets. Notably, the dense $Ca(H_2olz)$ framework **3** had released less than 25% of the drug at the same point. While these differences in solubility rates can be partly attributed to differences in pellet integrity, the improved resistance of the $Ca(H_2olz)$ materials to dissolution may nevertheless aid in preserving olsalazine as a solid throughout the upper gastrointestinal tract.

Multiple properties of the sodium- and calcium-olsalazine materials may contribute to the observed differences in drug release. For example, the differences in solubility are consistent with the expected trends for hard carboxylate donors with Na^+ versus Ca^{2+}.[17] Both the local and extended structure of the coordination solids may also play a role in governing the dissolution rates, since the three-dimensional material exhibits a distinct release profile compared to the other calcium-olsalazine materials. For instance, the number of water molecules coordinated to calcium may influence the rate of olsalazine dissociation required for hydrolysis. Because the materials resisted disintegration to different degrees, however, it is difficult to deconvolute the specific effects of crystal structure with that of other macroscopic or mechanical properties of the materials when compressed into a pellet without excipients.

Historically, sodium has been used far more frequently than other metal cations in drug formulation, largely due to its tendency to increase the solubility of an active pharmaceutical ingredient over its free acid form.[18] In this work, we show that calcium can be used to synthesize new solid-state architectures that may further optimize the performance of an existing drug by refining its release rates and solubility under physiological conditions. The slow-release properties of the $Ca(H_2olz)·xH_2O$ (x = 0, 2, 4) coordination solids may provide advantages over the commercial $Na_2(H_2olz)$ formulation by reducing the side effects associated with soluble olsalazine in the small intestine.

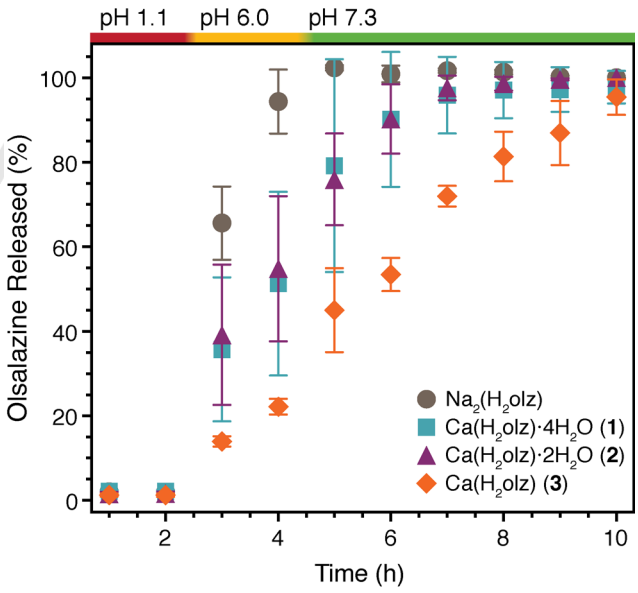

Figure 5. Release of olsalazine from $Na_2(H_2olz)$ (gray circles) and $Ca(H_2olz)·xH_2O$ materials under simulated gastrointestinal conditions (x = 4, 2, 0, denoted by teal squares, purple triangles, and orange diamonds, respectively). Error bars represent standard deviation across three independent data sets, where the quantity of olsalazine in solution was measured spectroscopically (λ = 360 nm). The release media were changed throughout the study to emulate the typical pH and transit times of a pill passing through the stomach (pH 1.1, 2 h, red), small intestine (pH 6.0, 2 h, yellow), and colon (pH 7.3, 6 h, green).

Acknowledgements

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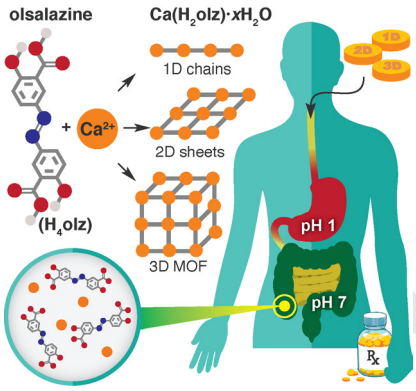
Keywords: drug delivery • calcium • metal-organic frameworks • coordination polymers • crystal engineering

1 C. Campregher and C. Gasche, *Best. Pract. Res. Clin. Gastroenterol.*, 2011, **25**, 535.
2 R. Karagozian and R. Burakoff, *Ther. Clin. Risk Manag.*, 2007, **3**, 893.
3 T. Sousa, V. Yadav, V. Zann, A. Borde, B. Abrahamsson and A. W. Basit, *J. Pharm. Sci.*, 2014, **103**, 3171.
4 P. R. Mudireddy, W. Blonski and G. R. Lichtenstein, in *Medical Therapy of Ulcerative Colitis*, Springer New York, New York, NY, 2014, pp. 69–99.
5 S. S. Rao, S. A. Dundas, C. D. Holdsworth, P. A. Cann, K. R. Palmer and C. L. Corbett, *Gut*, 1989, **30**, 675.
6 (*a*) H. Sandberg-Gertzén, G. Järnerot and W. Kraaz, *Gastroenterology*, 1986, **90**, 1024; (*b*) S. Meyers, D. B. Sachar, D. H. Present and H. D. Janowitz, *Gastroenterology*, 1987, **93**, 1255; (*c*) S. P. Travis, C. Tysk, H. J. De Silva, H. Sandberg-Gertzén, D. P. Jewell and G. Järnerot, *Gut*, 1994, **35**, 1282.
7 K. A. Kles, S. R. Vavricka, J. R. Turner, M. W. Musch, S. B. Hanauer and E. B. Chang, *Inflamm. Bowel Dis.*, 2005, **11**, 253.
8 (*a*) K. J. Goerg, R. Wanitschke, P. H. Diehl and K. H. Meyer zum Büschenfelde, *Gut*, 1988, **29**, 336; (*b*) R. Pamukcu, S. B. Hanauer and E. B. Chang, *Gastroenterology*, 1988, **95**, 975; (*c*) D. A. Chawla, P. I. Karl, R. N. Reich, G. Narasimhan, G. A. Michaud, S. E. Fisher and B. L. Schneider, *Dig. Dis. Sci.*, 1995, **40**, 943–948.
9 (*a*) J. Lai, L.-Q. Wang, K. Tu, C. Zhao and W. Sun, *Macromol. Rapid Commun.*, 2005, **26**, 1572. (*b*) X. Li, J. Li, Y. Gao, Y. Kuang, J. Shi and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 17707.
10 (*a*) Z. Ma and B. Moulton, *Coord. Chem. Rev.*, 2011, **255**, 1623; (*b*) P. Horcajada, R. Gref, T. Baati, G. Maurin, P. Couvreur, G. Férey, R. E. Morris and C. Serre, *Chem. Rev.*, 2012, **112**, 1232; (*c*) C.-Y. Sun, C. Qin, X.-L. Wang and Z.-M. Su, *Expert Opin. Drug Deliv.*, 2013, **10**, 89; (*d*) C. He, D. Liu and W. Lin, *Chem. Rev.*, 2015, **115**, 11079; (*e*) W. Cai, C. C. Chu, G. Liu and Y. X. J. Wáng, *Small*, 2015, **11**, 4806; (*f*) M. Rother, M. G. Nussbaumer, K. Renggli and N. Bruns, *Chem. Soc. Rev.*, 2016, **45**, 6213–6249.
11 (*a*) A. C. McKinlay, R. E. Morris, P. Horcajada, G. Férey, R. Gref, P. Couvreur and C. Serre, *Angew. Chem. Int. Ed.*, 2010, **49**, 6260; (*b*) S. Domingos, V. André, S. Quaresma, I. C. B. Martins, M. F. Minas da Piedade and M. T. Duarte, *J. Pharm. Pharmacol.*, 2015, **67**, 830; (*c*) S. Rojas, T. Devic and P. Horcajada, *J. Mater. Chem. B*, 2017, **5**, 2560.
12 (*a*) P. C. Andrews, G. B. Deacon, C. M. Forsyth, P. C. Junk, I. Kumar and M. Maguire, *Angew. Chem. Int. Ed.*, 2006, **45**, 5638; (*b*) S. R. Miller, D. Heurtaux, T. Baati, P. Horcajada, J.-M. Grenèche and C. Serre, *Chem. Commun.*, 2010, **46**, 4526; (*c*) E. H. H. Chow, F. C. Strobridge and T. Friščić, *Chem. Commun.*, 2010, **46**, 6368; (*d*) D. Liu, S. A. Kramer, R. C. Huxford-Phillips, S. Wang, J. Della Rocca and W. Lin, *Chem. Commun.*, 2012, **48**, 2668; (*e*) H. Su, F. Sun, J. Jia, H. He, A. Wang and G. Zhu, *Chem. Commun.*, 2015, **51**, 5774; (*f*) L. Cooper, T. Hidalgo, M. Gorman, T. Lozano-Fernández, R. Simón-Vázquez, C. Olivier, N Guillou, C. Serre, C. Martineau, F. Taulelle, D. Damasceno-Borges, G. Maurin, Á. González-Fernández, P. Horcajada and T. Devic, *Chem. Commun.*, 2015, **51**, 5848; (*g*) C. Tamames-Tabar E. Imbuluzqueta, N. Guillou, C. Serre, S. R. Miller, E. Elkaim, P. Horcajada and M. J. Blanco-Prieto, *CrystEngComm*, 2015, **17**, 456; (*h*) T. Hidalgo, L. Cooper, M. Gorman, T. Lozano-Fernández, R. Simón-Vázquez, G. Mouchaham, J. Marrot, N. Guillou, C. Serre, P. Fertey, Á. González-Fernández, T. Devic and P. Horcajada, *J. Mater. Chem. B*, 2017, **5**, 2813.
13 (*a*) Y.-Z. Tang, Y.-H. Tan, S.-H. Chen, Y.-W. Chao and P. Wang, *J. Coord. Chem.*, 2008, **61**, 1244; (*b*) D. J. Levine, T. Runčevski, M. T. Kapelewski, B. K. Keitz, J. Oktawiec, D. A. Reed, J. A. Mason, H. Z. H. Jiang, K. A. Colwell, C. M. Legendre, S. A. FitzGerald and J. R. Long, *J. Am. Chem. Soc.*, 2016, **138**, 10143
14 (*a*) P. C. Dietzel, R. Blom and H. Fjellvåg, *Z. Anorg. Allg. Chem.*, 2009, **635**, 1953; (*b*) A. E. Platero-Prats, V. A. de la Peña-O'Shea, N. Snejko, Á. Monge and E. Gutiérrez-Puebla, *Chem. Eur. J.*, 2010, **16**, 11632; (*c*) P.-C. Liang, H.-K. Liu, C.-T. Yeh, C.-H. Lin and V. Zima, *Cryst. Growth Des.*, 2011, **11**, 699; (*d*) S. R. Miller, P. Horcajada and C. Serre, *CrystEngComm*, 2011, **13**, 1894; (*e*) M. Mazaj, G. Mali, M. Rangus, E. Žunkovič, V. Kaučič and N. Zabukovec Logar, *J. Phys. Chem. C*, 2013, **117**, 7552.
15 (*a*) M. Lipkin, E. Friedman, S. J. Winawer and H. Newmark, *Cancer Res.*, 1989, **49**, 248; (*b*) S. A. Lamprecht and M. Lipkin, *Nat. Rev. Cancer*, 2003, **3**, 601; (*c*) M. A. A. Schepens, A. J. Schonewille, C. Vink, E. M. van Schothorst, E. Kramer, T. Hendriks, R.-J. Brummer, J. Keijer, R. van der Meer and I. M. J. Bovee-Oudenhoven, *J. Nutr.*, 2009, **139**, 1525.
16 D. K. Bučar, R. W. Lancaster and J. Bernstein, *Angew. Chem. Int. Ed.*, 2015, **54**, 6972–6993.
17 (*a*) S. H. Wan, P. Pentikainen and D. L. Azarnoff, *J. Pharmacokinet. Biopharm.*, 1974, **2**, 1; (*b*) Z. T. Chowhan, *J. Pharm. Sci.*, 1978, **67**, 1257; (*c*) B. D. Anderson and R. A. Conradi, *J. Pharm. Sci.*, 1985, **74**, 815; (*d*) K. R. Morris, M. G. Fakes, A. B. Thakur, A. W. Newman, A. K. Singh, J. J. Venit, C. J. Spagnuolo and A. T. M. Serajuddin, *Int. J. Pharm.*, 1994, **105**, 209; (*e*) R. T. Forbes, P. York and J. R. Davidson, *Int. J. Pharm.*, 1995, **126**, 199; (*f*) J.-B. Arlin, A. J. Florence, A. Johnston, A. R. Kennedy, G. J. Miller and K. Patterson, *Cryst. Growth Des.*, 2011, **11**, 1318.
18 G. S. Paulekuhn, J. B. Dressman and C. Saal, *J. Med. Chem.*, 2007, **50**, 6665.

Entry for the Table of Contents

COMMUNICATION

Visceral reaction: Olsalazine, an anti-inflammatory drug used to treat ulcerative colitis, can cause side effects that arise from early release in the small intestine. To mitigate this, calcium-olsalazine coordination solids were synthesized that resist dissolution at low pH and gradually release olsalazine at neutral pH. The calcium materials also dissolved more slowly than the commercial sodium salt, making them potential candidates for improved olsalazine delivery to the colon.



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