

Selective Growth and Distribution of Crystalline Enantiomers in Hydrogels

Rositza I. Petrova and Jennifer A. Swift*

Contribution from the Department of Chemistry, Georgetown University,
37th and "O" Streets NW, Washington, DC 20057-1227

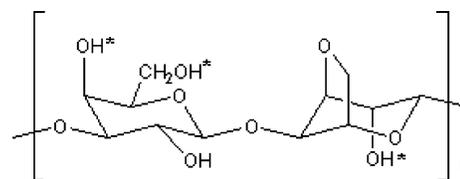
Received October 24, 2003; E-mail: jas2@georgetown.edu

Abstract: The crystallization of sodium chlorate (NaClO_3) is a classic example of spontaneous chirality, since it is achiral in solution but adopts a chiral form in the solid state. While crystal growth of NaClO_3 from pure aqueous solutions yields a 50:50 statistical distribution of *d*- and *l*-crystals, large enantiomeric excesses of either *d*- and *l*-crystals can be achieved by crystal growth in agarose gel, a naturally occurring chiral polysaccharide. The influence of gel density (0.1–0.75 wt %), temperature, and the diffusion of cosolvents on crystal distribution was discerned from statistical data obtained from 752 gel-mediated crystallization experiments yielding 12,384 individual crystals. These studies demonstrate that the magnitude and direction of the bias can be selectively engineered toward either *d*- or *l*-forms by changing the gelation conditions. Aqueous agarose gels infused with 48 wt % NaClO_3 at 6 °C, favored the growth of *d*- NaClO_3 crystals, with ee's reaching 22% at the highest gel concentrations. Crystal growth under methanol diffusion favored deposition of the opposite enantiomorph, *l*- NaClO_3 . The bias in the crystal distribution is enhanced at higher temperatures. Aqueous gels at 24 °C infused with methanol cosolvent favored *l*- NaClO_3 , with ee's reaching 53%. The changing magnitude and direction of the enantiomorph bias can be ascribed to differences in the agarose conformation and intermolecular interactions between the gel and crystal surfaces that inhibit the formation of the two enantiomers to different extents.

Introduction

Gel media has been used for over a century¹ to grow inorganic, organic,^{2–4} and macromolecular^{5,6} crystals with high optical quality and appreciable size. The improved physical characteristics of gel-grown crystals (i.e., larger, fewer defects) over solution-grown ones is usually ascribed to the suppression of convection currents, sedimentation, and nucleation afforded by the more viscous media. While it is generally accepted that the gel does not exert major mechanical forces on the crystals grown within, the influence of chemical forces between the gel and crystal have received far less attention to date. We hypothesized that if sufficient crystal–gel interactions exist, they might provide a reasonable bias to alter the crystallization rates of otherwise equienergetic *d*- and *l*-enantiomorphs. In the present “proof of concept” study, we demonstrate that crystal growth in chiral agarose gel media can be used as a means to preferentially bias the distribution of sodium chlorate (NaClO_3), and that through slight modification of the gelation conditions, both the magnitude and the direction of the bias can be altered.

Agarose is a linear copolymer composed of alternating (1→4) linked 3,6-anhydro- α -L-galactose and (1→3) linked β -D-galactose that forms thermoreversible gels in aqueous solution.



agarose

In dilute solutions, or above the gelation temperature, agarose is believed to exist in a single-coil conformation.^{7–11} Upon cooling, the solution undergoes a liquid–liquid phase transition through spinodal demixing, which leads to the formation of low- and high-concentration domains. In the high concentration regions, conformational changes lead to the formation of double helices, which at the gelling temperature form rodlike fibers with a diameter of a few nanometers.^{8,12} Agarose gelation is temperature dependent, but usually requires concentrations of 0.1% or higher.

- (1) Hensch, H. K. *Crystals in Gels and Liesegang Rings*; Cambridge University Press: New York, 1988.
- (2) Yaghi, O. M.; Li, G.; Li, H. *Chem. Mater.* **1997**, *9*, 1074–1076.
- (3) Etter, M. C.; Jahn, D. A.; Donahue, B. S.; Johnson, R. B.; Ojala, C. J. *Cryst. Growth* **1986**, *76*, 645–655.
- (4) Desiraju, G. R.; Curtin, D. Y.; Paul, I. C. *J. Am. Chem. Soc.* **1977**, *99*, 6148.
- (5) McPherson, A. *Crystallization of Biological Macromolecules*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999.
- (6) Robert, M. C.; Lefaucheux, F. *J. Cryst. Growth* **1988**, *90*, 358–367.

- (7) Pines, E.; Prins, W. *Macromolecules* **1973**, *6*, 888–895.
- (8) Djabourov, M.; Clark, A. H.; Rowlands, D. W.; Ross-Murphy, S. B. *Macromolecules* **1989**, *22*, 180–188.
- (9) Emanuele, A.; Di Stefano, L.; Giacomazza, D.; Trapanese, M.; Palma-Vittorelli, M. B.; Palma, M. U. *Biopolymers* **1991**, *1991*, 859–868.
- (10) Manno, M.; Emanuele, A.; Martorana, V.; Bulone, D.; San Biagio, P. L.; Palma-Vittorelli, M. B.; Palma, M. U. *Phys. Rev. E* **1999**, *59*, 2222–2229.
- (11) Kirkpatrick, F. H. *Curr. Commun. Cell Mol. Biol.* **1990**, *1*, 9–22.
- (12) Whytock, S.; Finch, J. *Biopolymers* **1991**, *31*, 1025–1028.

The molecular-level tertiary structure of three single- and one double-helical structures of agarose, all left-handed, was predicted by Kouwijzer and Perez,¹³ based on previous X-ray data and optical rotation calculations.^{14,15} Their model asserts wide-diameter (0.45 nm) helices with three-fold symmetry and a 1.90 nm pitch. The two chains of the double helix are related by a translation of 9.5 Å along the helical axis. The expected 30–45% water content in the gels found in these structures agrees with their gel-forming behavior. In the double helix conformation, three of the hydroxyl groups are projected on the exterior of the helix (depicted with an *), where they are available to interact via hydrogen-bonding to other helices, solvent and/or solute molecules. Further experimental studies on the effect of various salts on the coil–helix transition of agarose have demonstrated that the presence of organic solvents or anionic cosolutes can stabilize or destabilize the gel state by preferential adsorption of either denaturant or water molecules to the agarose macromolecule.^{16–19} The presence of such cosolutes or solvents also likely alters the conformation of the agarose macromolecule.

The model crystal system employed in this study is sodium chlorate (NaClO₃). Solutions of NaClO₃ are achiral, but the slow evaporation of supersaturated aqueous solutions yields well-developed rectangular or cubic crystalline prisms of the enantiomorphous space group *P*2₁3.²⁰ The *d*- and *l*-crystal forms are easily distinguished by their ability to rotate linearly polarized light by $\sim\pm 3^\circ/\text{mm}$. Although the *d*:*l* ratio of crystals obtained in a single crystallization experiment may deviate from the expected 50:50, the *statistical average* of many crystal growth experiments converges at 50:50. This was first demonstrated as early as 1898 by Kipping and Pope,²¹ and has been subsequently been repeated by several other research groups.

A variety of approaches to alter the distribution of NaClO₃ enantiomorphs have been pursued,²² including stirring,^{23,24} exposure to β radiation,²⁵ and the addition of chiral seed crystals²⁶ or other chiral impurities.^{21,27} Large *ee*'s can be obtained in a single experiment under stirring conditions, an observation which can be explained by secondary nucleation effects which produce homochiral seeds.²⁸ However, under such conditions it is impossible to predict the direction of the bias *a priori*, because the formation of an initial *l*- or *d*-crystal is equally probable. To selectively bias the distribution of enantiomers toward preferentially one form, the intentional addition

of homochiral impurities can be an effective means. In the original 1898 experiments, crystal growth in the presence of high concentrations (200 g/L) of glucose favored the crystallization of *l*-NaClO₃, while galactitol impurities showed a slight bias toward *d*-NaClO₃. Niedermaier and Schlenk²⁷ have additionally shown that *D*-mannitol impurities favor *d*-NaClO₃ while *D*-sorbitol favors *l*-NaClO₃.

The fundamental question in the present study was to determine whether the spontaneous resolution of NaClO₃ could be influenced by growth in a chiral gel medium. Unlike stirred solutions, any bias obtained from gel-mediated growth should favor the same enantiomer over a statistically significant number of crystallizations. Secondary nucleation effects should also not be a significant factor in gel matrixes, where crystallization rates are generally slower than that of pure solution and where random collisions between molecules/ions are minimized. The concentration of gelling component in most thermoreversible gels such as agarose (i.e. <1.0 wt %) is also at least an order of magnitude smaller than that used in previous studies with chiral molecular impurities.

Experimental Section

Materials. Sodium chlorate, 99+% (Aldrich) and agarose (Type I, Sigma) with sulfate content of 0.06% were used without further purification. The same batch of agarose was used for all experiments. Water was purified by passage through two Barnstead deionizing cartridges followed by distillation.

Gel Preparation. Thermoreversible agarose gels were formed in standard test tubes with a diameter of 25 mm by heating an aqueous suspension of sodium chlorate (48 wt %) and the gelling substance (0.10–0.75 wt %) to 80 °C in a water bath until clear solutions were obtained. The total gel volume was 20 mL. The hot solutions were covered with Parafilm, slowly cooled to room temperature over a period of an hour, and then placed in a 6 °C refrigerator for 2 h. After that time, some of the gels remained in the 6 °C refrigerator, while others were removed and allowed to warm to room temperature 24 ± 1 °C. Over a period of a few days, crystals of visible size (2–10 mm edge length) grew in most of the 6 °C aqueous gels. More rapid crystallization in 6 °C gels was encouraged by the addition and diffusion of ~ 5 mL methanol into gels after 16 hours. Only a small fraction of the 24 °C gels produced visible crystals over a period of weeks to months, while the majority of gels remained optically transparent and failed to show crystal growth. When methanol was diffused into optically transparent gels at either 6 or 24 °C, crystallization occurred over a period of several hours to days. Crystals were harvested from the gel matrix manually using tweezers and their individual handedness determined using an OLYMPUS BX50 polarizing microscope.

Crystal Growth from Solution. Growth experiments performed in pure aqueous solution establish that the starting NaClO₃ material was not contaminated with other chiral impurities. In covered Petri dishes with a diameter of 10 cm and a total volume of 20 mL, NaClO₃ crystals were grown from 52 wt % aqueous solution and yielded a statistical distribution of enantiomorphs with $W_L = 50.14 \pm 1.64\%$. Identical experiments were also performed with 1 wt % *D*-galactose included as an additive.

Statistics. Nucleation biases were assessed by conventional statistical methods for each of the different solution and gel growth conditions. Equations used to calculate the weighted percentage of *l*-NaClO₃ crystals (W_L) and the standard deviation (σ) were obtained from reference 29.

Gelation Temperature Determination. The optical rotation measurements were performed on a Rudolph Instruments polarimeter

- (13) Kouwijzer, M.; Perez, S. *Biopolymers* **1998**, *46*, 11–29.
- (14) Foord, S. A.; Atkins, E. D. T. *Biopolymers* **1989**, *28*, 1345–1365.
- (15) Schafer, S. E.; Stevens, E. S. *Biopolymers* **1995**, *36*, 103–108.
- (16) San Biagio, P. L.; Newman, J.; Madonia, F.; Palma, M. U. *Chem. Phys. Lett.* **1989**, *1989*, 477–483.
- (17) Bullone, D.; Emanuele, A.; San Biagio, P. L. *Biophys. Chem.* **1999**, *77*, 1–8.
- (18) Piculell, L.; Nilsson, S. *J. Phys. Chem.* **1989**, *93*, 5596–5601.
- (19) Piculell, L.; Nilsson, S. *J. Phys. Chem.* **1989**, *93*, 5602–5611.
- (20) Ramachandran, G. N.; Chandrasekaran, K. S. *Acta Crystallogr.* **1957**, *10*, 671–675.
- (21) Kipping, F. S.; Pope, W. J. *J. Chem. Soc. (London) Trans.* **1898**, *73*, 606–617.
- (22) Pagni, R. M.; Compton, R. N. *Cryst. Growth Des.* **2002**, *2*, 249–253.
- (23) Kondepudi, D. K.; Kaufman, R. J.; Singh, N. *Science* **1990**, *250*, 975–976.
- (24) Martin, B.; Tharrington, A.; Wu, X.-I. *Phys. Rev. Lett.* **1996**, *77*, 2826–2829.
- (25) Mahurin, S.; McGinnis, M.; Bogard, J. S.; Hulett, L. D.; Pagni, R. M.; Compton, R. N. *Chirality* **2001**, *13*, 636–640.
- (26) Vogl, O.; Qin, M.; Bartus, J.; Jaycox, G. D. *Monatsh. Chem.* **1995**, *126*, 67–73.
- (27) Niedermaier, T.; Schlenk Jr., W. *Chem. Ber.* **1972**, *105*, 3470–3478.
- (28) McBride, J. M.; Carter, R. L. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 293–295.

- (29) Bevington, P. R.; Robinson, D. K. *Data Reduction and Error Analysis for the Physical Sciences*, 3rd ed.; McGraw-Hill: Boston, 2003.

(DigiPol DP781) at $\lambda = 589$ nm in a water-jacketed optical cell with a path length of 10 cm. The cooling rate was 0.5 °C/min.

Results and Discussion

Discerning Gel Conditions. The outcome of any individual gel-growth experiment can be sensitive to a variety of factors including [NaClO₃], [agarose], temperature, time, impurities, and the procedure for gel preparation. Appropriate concentrations of NaClO₃ and agarose to be used in the study were therefore the first variables ascertained. All of the gel growth experiments described herein used a fixed [NaClO₃] = 48 wt %. If significantly higher solute concentrations were used, precipitation before gel setting can occur, while for solute concentrations significantly lower than 48 wt %, supersaturation conditions may not be met, and crystal growth is unlikely to occur. The agarose concentration range useful for crystal growth was also limited to 0.1–0.75 wt %. The least dense agarose gels (0.1%) exhibited near fluidlike viscosity, while agarose gel concentrations ≥ 1.0 wt % were often too dense to support the growth of millimeter-size crystals.

Optical rotation studies at variable temperatures revealed that the addition of NaClO₃ to agarose gel destabilizes the gelation process, as is the case with the addition of other ionic impurities. Helix formation in pure 0.50% agarose gels upon cooling occurs at 32 °C, and is accompanied by a large change in the optical rotation of the solution. As increasing concentrations of NaClO₃ are added to the agarose gel, the associated optical rotation change occurs at progressively lower temperatures, reaching 12 °C when [NaClO₃] = 48 wt %. To ensure that helix formation was allowed to occur in all samples used in this study, agarose gels containing NaClO₃ were prepared by heating an aqueous suspension of NaClO₃ and agarose to 80 °C until a clear solution was obtained, slow cooling to room temperature over a period of an hour, and additional cooling in a 6 °C refrigerator for 2 h. After that time, some of the gels remained at 6 °C, while others were subsequently allowed to warm to room temperature, 24 \pm 1 °C. Gels at 24 °C remained optically transparent throughout the warming process.

Crystal growth in agarose gels was examined at both 6 and 24 °C. The solubility of NaClO₃ in water at 10 and 25 °C is 46.67 and 50.1 wt %, respectively.³⁰ This means that while 6 °C gels are supersaturated with respect to NaClO₃, those at room temperature are slightly undersaturated. At 6 °C, NaClO₃ crystals of visible size spontaneously appeared in the majority of gels over a period of several days to weeks (Figure 1). When a few milliliters of methanol, a miscible cosolvent, was allowed to slowly diffuse into the 6 °C agarose gel matrix, crystals appeared over a period of just a few hours. In comparison, only a small percentage of the 24 °C gels spontaneously developed crystals over a period of weeks to months. However, when a few milliliters of methanol was diffused into 24 °C agarose gels, the solubility of NaClO₃ was sufficiently reduced to allow crystal growth to occur in all gels over a period of just a few hours. The methanol diffusion was applied to two different “classes” of room-temperature gels—those that were treated with methanol soon after warming to 24 °C and those that remained optically transparent at 24 °C for 45 days and only yielded crystals upon cosolvent addition. Under all gel growth conditions, crystals

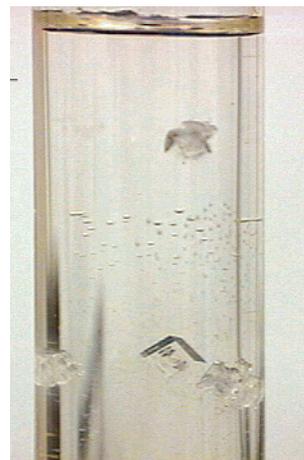


Figure 1. Photograph of typical agarose gel showing crystals of NaClO₃ grown within the matrix.

formed within the agarose matrix appear to be grown isolated from one another (i.e. not in clusters). Crystals were manually harvested from each gel matrix with tweezers when they reached sizes of ~ 2 –10 mm³. We note that in all experiments, the total mass of the crystals deposited is only a small fraction ($\sim 6.5\%$) of the original material initially dissolved within the gel.

Qualitative habit changes as a function of gel concentration in these systems were observed. NaClO₃ crystals adopt a cubic morphology determined by {100} faces when grown from pure aqueous solutions and from gels with [agarose] < 0.25 wt %. Under higher gel concentrations, once a cubic seed was formed (0.5–1 mm³), the crystals tended to grow faster along [111] and form dendrites or develop “hopper” shape, which is characteristic for a 2-D nucleation and growth along the edges.³¹ Similar morphologies have been observed for isomorphous NaBrO₃ crystals grown under agarose gel conditions.³² Under accelerated growth conditions with the addition of cosolvent, crystal dendrites are often first observed at the gel/methanol interface, and propagate into the gel bulk. In general, crystals grown from agarose appear somewhat cloudier than their solution-grown analogues. The presence of small quantities of agarose occluded in the crystal matrix was confirmed by TLC separation of the chlorate and agarose followed by the Molish test.³³

Enantiomer Distribution. Conclusions drawn from these experiments depend on data of a statistical nature. For each experimental condition (6 and 24 °C, with and without methanol diffusion), identical crystallization experiments were performed at each of four agarose concentrations ranging from 0.1 to 0.75%. Table 1 summarizes the crystal data obtained from all agarose-mediated growth experiments, while actual data tables for all individual experiments are provided in the Supporting Information. An identical number of control experiments in pure aqueous solution and in solutions containing 1.0% D-galactose impurity were also performed.

Enantiomorph biases were assessed for all experiments yielding two or more crystals using standard statistical methods. As not all experiments yielded the same number of crystals,

(30) Company, C. R. *CRC Handbook of Chemistry and Physics*, 81st ed.; CRC Press: Boca Raton, FL, 2001.

(31) Doxsee, K. M.; Chang, R. C.; Chen, E.; Myerson, A. S.; Huang, D. *J. Am. Chem. Soc.* **1998**, *120*, 585–586.

(32) Petrova, R. I.; Swift, J. A. *Cryst. Growth Des.* **2002**, *2*, 573–578.

(33) Robyt, J. F.; White, B. J. *Biochemical Techniques: Theory and Practice*; Brooks/Cole: Monterey, CA, 1987.

Table 1. NaClO₃ Crystals Grown from Agarose Gel under Various Conditions

[agarose]	# of Expts	# <i>l</i> crystals	# <i>d</i> crystals	Total # crystals
(Δ) 6°C				
[0.10]	40	178	219	397
[0.25]	40	154	128	282
[0.50]	40	95	159	254
[0.75]	40	91	143	234
(□) 6°C & methanol diffusion				
[0.10]	40	405	353	758
[0.25]	40	466	353	819
[0.50]	40	399	329	728
[0.75]	40	419	337	756
(▲) 24°C				
[0.10]	11	59	64	123
[0.25]	12	60	84	144
[0.50]	23	96	87	183
[0.75]	31	70	53	123
(●) 24°C & methanol diffusion				
[0.10]	40	655	723	1378
[0.25]	45	771	575	1346
[0.50]	52	563	323	886
[0.75]	58	734	326	1060
(■) 24°C & methanol diffusion				
[0.10]	40	488	424	912
[0.25]	40	401	348	749
[0.50]	40	426	174	600
[0.75]	40	499	153	652
TOTAL	752	7029	5355	12384

Gel conditions: (Δ) = 6°C, aqueous gel; (□) = 6°C, methanol cosolvent added after 16 hours; (▲) = 24°C, aqueous gel; (●) = 24°C, methanol cosolvent added soon after initial gel setting; (■) = 24°C, methanol cosolvent addition to gels which remained optically transparent for 45 days.

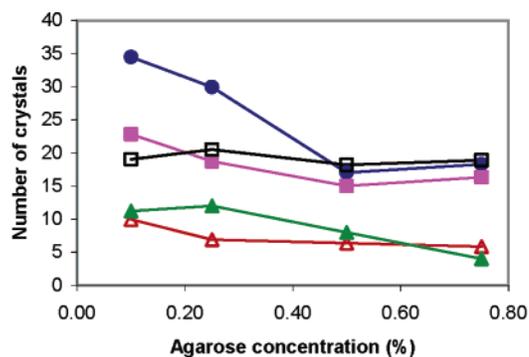
each experiment was weighted according to the total number of crystals grown. Statistics obtained using both weighted or unweighted mean percentages yield similar trends; however, weighted analyses provided a somewhat more accurate assessment, since they minimize the potential impact of a small number of experiments that yield just a few crystals. The equations used to calculate the weighted percentage of *l*-NaClO₃ crystals (W_L) and the standard deviation (σ) are as follows:²⁹

$$W_L = \frac{100 \sum L}{\sum (L + D)}$$

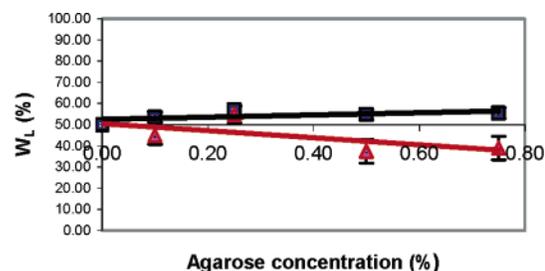
$$\sigma = \sqrt{\frac{1}{N-1} \left[\frac{\sum \{(L+D)(100L/(L+D))^2\}}{\sum (L+D)} - W_L^2 \right]}$$

where L - number of *l*-crystals; D - number of *d*-crystals, N - number of experiments.

Two trends emerged from the data sets obtained. First, under all experimental conditions, as the density of the agarose gel



(Δ) = 6°C, aqueous gel; (▲) = 24°C, aqueous gel; (□) = 6°C, methanol cosolvent added after 16 hours; (●) = 24°C, methanol cosolvent added soon after initial gel setting; (■) = 24°C, methanol cosolvent added to gels which remained optically transparent for 45 days.

Figure 2. Dependence of the average number of crystals generated per experiment on agarose concentration.

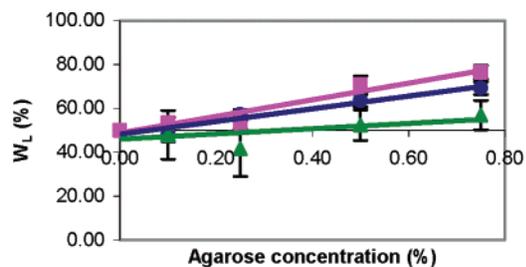
Gel conditions: (Δ) = 6°C, aqueous gel; (□) = 6°C, with diffusion of methanol cosolvent.

Figure 3. Dependence on the weighted mean percentage of the *l*-enantiomorph (W_L) as a function of [agarose] for gels at 6°C.

increases, fewer crystals are formed (Figure 2). This observation is consistent with other crystal growth experiments in viscous media, and suggests that the gel functions overall more like an inhibitor than a growth templating agent. The total number of crystals grown under methanol diffusion conditions is also generally larger than those grown from pure aqueous gels. That a majority of the room-temperature gels developed crystals over a 45-day period, suggests that undissolved NaClO₃ seed crystals are not an important factor in growth under these gel conditions. All crystals are presumed to grow from nuclei formed within the gel matrix.

A more interesting trend is observed in the distribution of the *l*- and *d*-enantiomorphs grown inside the gel matrix. Agarose-mediated crystal growth at 6°C showed a slight bias toward the formation of *d*-NaClO₃ (Figure 3). The magnitude of the statistical bias grows nearly linearly with respect to the agarose concentration, reaching $ee = 22\%$ under 0.75 wt % gel conditions. Crystal growth accelerated by diffusion of methanol into identical 6°C agarose matrixes, resulted in a surprising reversal of the earlier trend and an increasing bias toward the formation of the opposite enantiomer, *l*-NaClO₃ ($W_L = 55.42 \pm 2.49\%$ for 0.75 wt % gels).

In growth from both aqueous gels and those subjected to methanol diffusion, the observed bias must be ascribed to a difference in the intermolecular interactions between the gel and chiral surfaces of either *d*- or *l*-crystal enantiomorphs. The observation that fewer crystals are grown at higher gel concen-



Gel Conditions: (\blacktriangle) = 24 °C, aqueous gel; (\bullet) = 24 °C, methanol cosolvent added soon after initial gel setting; (\blacksquare) = 24 °C, methanol cosolvent added to gels which remained optically transparent for 45 days.

Figure 4. Dependence on the weighted mean percentage of the *l*-enantiomorph (W_L) as a function of [agarose] for gels at 24 °C.

trations, suggests that agarose in water is somewhat more effective at inhibiting the formation of *l*-nuclei and/or limiting the growth rate of *l*-crystals relative to the *d*-form. When small quantities of a miscible cosolvent are allowed to slowly diffuse into the aqueous agarose gel, some solvent molecules will inevitably replace water molecules hydrogen bonded to the exterior of the gel's helices. Alteration of the gel's conformation in response to the more hydrophobic environment will lead to interactions at the gel–crystal interface that are no longer identical to those in pure water. Unfortunately, establishing a more detailed picture of the molecular geometries and interactions at the gel–crystal interface is quite challenging both experimentally and computationally, because the gel always retains some degree of conformational flexibility and because water and/or methanol molecules likely play an integral role in the structure and/or energetics of the chiral interface. However, it is clear that by addition of an achiral solvent to the gel mixture, one can reproducibly “engineer” the direction of the bias toward *d*- or *l*-forms of NaClO₃.

To discern whether the direction and/or magnitude of the bias changes as a function of temperature, analogous experiments were also performed at 24 °C (Figure 4). Reliable statistics for gel-mediated growth at 24 °C under pure aqueous conditions were difficult to obtain, because only a small fraction of gels support spontaneous crystal growth. Interpretation of the admittedly limited statistics available for enantiomers grown under these conditions does not provide any clear indication of a significant bias toward either *d*- or *l*-forms. All measurements fall within experimental error of a 50:50 distribution.

However, better statistics can more readily be obtained in the case of methanol diffusion, which can be used to induce crystallization even at 24 °C. Methanol diffusion was applied to two types of agarose gels: (1) freshly prepared gels and (2) gels that remained optically transparent and otherwise failed to yield crystals after 45 days at room temperature. Both of these gel types exhibited a clear bias toward *l*-NaClO₃ crystals, a bias that grew linearly with increasing gel concentrations. Interestingly, the magnitude of the bias for fresh 0.75 wt % agarose was significantly larger at 24 °C ((blue \bullet) $W_L = 69.25 \pm 2.89\%$) than in the analogous diffusion experiments performed at 6 °C ((black \square) $W_L = 55.42 \pm 2.49\%$). Gels aged for 45 days (red \blacksquare) exhibited slightly stronger biases than the fresh gels, with ee's as high as 53% ($W_L = 76.53 \pm 3.15\%$).

One possible reason for the magnified bias at elevated temperatures may be due to differences in the initial gel stiffness. For gels of a given concentration, lower temperatures and/or

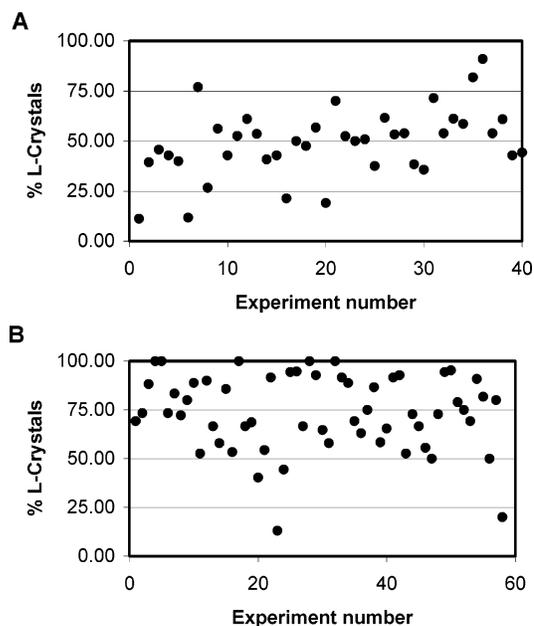


Figure 5. Scattergrams of the percentage *l*-crystals grown for each of the experiments performed under diffusion of methanol at 24 °C with two different gel concentrations: (A) 0.10 wt % agarose; (B) 0.75 wt % agarose.

the absence of methanol leads to qualitatively stiffer agarose gels. Others have suggested that increased gel stiffness correlates with a relatively higher concentration of double helices compared to single helices.¹⁶ Differences in the stiffness of the gels may also influence the rate at which the agarose helices can alter their conformation in response to the changing solution conditions when methanol is introduced.

For experiments that require statistical analysis such as these, it is also reasonable to assess the variability of the data collected under identical experimental conditions. Scattergrams in Figure 5 give a sense for the variability in the distribution of enantiomers obtained in each of the individual crystallization experiments performed under an identical set of conditions. The % *l*-NaClO₃ obtained from each of the 0.10 wt % agarose gels subjected to methanol diffusion at 24 °C (plot A) are mostly scattered around the 50%-line (W_L value of $47.53 \pm 2.64\%$), though the percentage of *l*-crystals from any individual experiment ranges from 11.11 to 90.91%. Under higher 0.75 wt % agarose concentrations, the clear bias toward *l*-NaClO₃ is readily apparent from the analogous scattergram (plot B). The variability in the data is also decreased, with 54/58 experiments showing a preference for *l*-NaClO₃, and only 4/58 showing a preference for *d*-NaClO₃.

The modest differences in ee's obtained from gels of different ages is more difficult to explain, as we do not presently have any information on how aging affects the gel structure. Considering the destabilizing effect of NaClO₃ on agarose gel and the presence of methanol, we sought to examine whether there is any correlation between W_L and the time the crystals were harvested from the gel (Figure 6). However, no discernible trend in variation of W_L as a function of time during the period from 24 to 96 h was observed. Given the very similar W_L values obtained for crystals grown under diffusion of methanol in freshly prepared gels and in 45-day-old gels (Figure 4), any changes in agarose structure that might occur over this time scale do not appear to have a discernible effect on the crystallization of sodium chlorate.

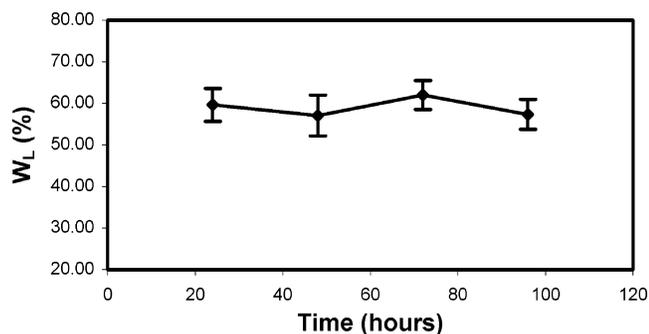


Figure 6. W_L vs time for crystals grown under diffusion of methanol at 24 °C. [agarose] = 0.25 wt % [NaClO₃] = 48 wt %.

Conclusions

In summary, we have demonstrated that the statistical distribution of NaClO₃ crystals can be significantly biased from the expected 50:50 *l:d* ratio by growth in agarose gel media. The extent of the bias is clearly dependent on a number of factors, which we have attempted to elucidate systematically in order to develop gel methods as a rational means to influence crystallization events. Crystal growth in 6 °C aqueous agarose gels shows a modest bias toward *d*-NaClO₃, but the direction of the bias can be shifted toward the *l*-enantiomer by the diffusion of methanol cosolvent. Elevating the gel temperature to 24 °C can further enhance the magnitude of the bias toward significantly higher *ee*'s.

Although the conformation of agarose in water and water/methanol solutions is presumably different, a molecular view

of the origin of the chiral bias remains somewhat ambiguous. Agarose is chiral on both the primary (monomer) and tertiary (helices) length scales. Our attempts to grow NaClO₃ from solutions containing 1.0 wt % D-galactose yielded no discernible bias in the enantiomorph distribution ($W_L = 51.11 \pm 1.88\%$). However, this does not prove that helices are the key chiral structure-directing features, as it is known that polymeric additives can sometimes bias chiral crystal nucleation rates more efficiently than equivalent concentrations of monomers.³⁴ Our ongoing studies focus on improving the material yield obtained from gel crystallizations and on the application of gel methods to more specialized chemical resolutions.

Acknowledgment. We are grateful for the financial support provided by the Henry Luce Foundation, the ACS-Petroleum Research Fund (36457-G5), and the NSF through a Career Award (DMR-0093069). We additionally thank B. Cracchiolo and the students of CHEM 118 (Spring 2001) for their contribution to this work.

Supporting Information Available: Tables of crystal growth data for each set of experimental conditions presented in Table 1, and tables of crystal growth data from the solution control experiments and kinetics experiments (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA0392263

(34) Zbaida, D.; Weissbuch, I.; Shavit-Gati, E.; Addadi, L.; Leiserowitz, L.; Lahav, M. *React. Polym.* **1987**, *6*, 241–253.