Self-Assembly of Folic Acid in Aqueous Media

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The self-assembly of folic acid solutions at low concentrations, which has implications in its possible use as a drug delivery carrier, is presented. X-ray diffraction (XRD) is used to show that folic acid has ordered structures in solution at concentrations lower than 1 wt %, but only in its ionized state. Various microscopy techniques and rheological studies are used to show existence of different phases of the ordered structures in the solution phase as well as the properties of this complex fluid. In conjunction with semiempirical calculations and molecular dynamics simulations, the XRD studies help to understand the mechanisms of formation of the ordered phase as well as the structure in solution.
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Introduction

Drug delivery strategies form an integral part of therapeutics of a wide variety of drugs including anticancer drugs, protein, and RNA-based drugs as well as some key small molecule drugs. While polymers have been explored quite extensively, in the last two decades micelles formed from amphiphiles of different kinds have also been designed to form emulsions and vesicles carrying drugs. In the last decade, even more highly ordered sets of materials have presented rather interesting results such as versatility of drug encapsulation, high encapsulation yields, and controlled release rates. One such set of materials is chromonics.

Chromonics are a group of molecules that are understood to form ordered phases driven by enthalpic interactions[1] between the particles rather than entropic.[2–5] They are made up of aromatic complexes, sometimes linked by short chains with hydrophilic functional groups. Examples of chromonics materials include a variety of drugs and dyes.[6–13] The aromatic groups of these molecules drive these molecules to form ordered structures such that the aromatic groups face each other—the mechanisms and applications have been presented in a recent review.[14] Such ordered structures are observed to encapsulate other molecules—notably similar structured aromatic dyes but also other molecules such as nonaromatic drugs and proteins.

The ability of chromonic molecules to form ordered structures such that they can form single-phase solutions with drug molecules are of primary interest to this research. We are interested in the mechanisms and structure of the ordered assemblies, and impact of perturbation in pH, or concentration, because these aspects are important to formation of drug carriers and the processing of such solutions.

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In the past, some significant studies have been undertaken in presenting the formation of ordered structures using *n*-methyl imidazol-based chromonics molecules[15] as well as their ability to encapsulate and release a broad set of drugs. In this study, we focus on folic acid. Folic acid is a molecule (Figure 1) that shows self-assembled structures; past literature reports self-assembly only at relatively high concentrations[16–18] (compared to other chromonic structures) of over 20 wt %. The interest in folic acid self-assembly is driven by two reasons. One, the structure of folic acid has similarities with other chromonic molecules—resonant aromatic rings with hydrophilic functional groups suggest chromonic-like self-assemblies. This implies that folic acid molecules should assemble owing to enthalpic reasons and, hence, should show no (or extremely low) threshold for assembly. These molecules should show hierarchical structures where stacks of folic acid assemblies also organize into higher-order structures. These implications have significant bearing on the ability of a self-assembled structure to encapsulate, protect, and deliver through controlled release, guest molecules of interest.

The second reason is that folic acid is a molecule that is commonly ingested by humans—as part of our daily diets in vegetables and fruits and as dietary supplements. Thus, it has low risk of toxicity or other side effects. The molecule is biocomaptible, especially with human systems. Moreover, there are claims that folic acid can be used in targeting strategies for anticancer therapeutics. These reasons provide a strong motivation to study self-assembly of folic acid in greater detail.

Key previous work on self-assembly of folic acid comes from Gottarelli.[16,17] Focusing on self-assembly of folic acid and other similarly structured biomolecules and their derivatives,[18–21] these studies show self-assembly of folic acid at weight fractions higher than 25%, where they see hexagonal phases. They find that the onset of long-range order is at 20% when sodium salts are added; they see cholesteric phases and subsequently, hexagonal phases at about 23% of the folate component by weight.

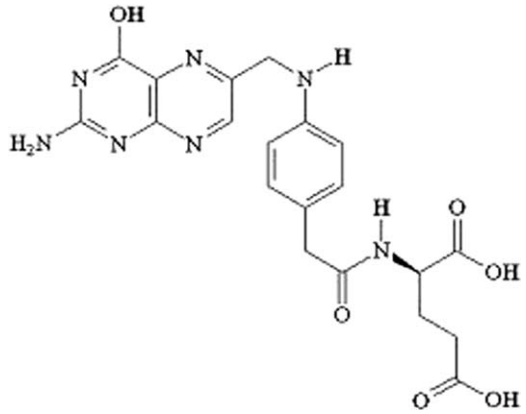

Figure 1. Molecular structure of folic acid.

In this article, we are interested in self-assembly of low to moderate concentrations of folic acid (0.01–25 wt %) owing to practical implications of self-assembly at these low to moderate concentrations. The use of these complex fluids in numerous applications (from formation of optically interesting structures with other dyes or by themselves, as coatings, in forming surface coatings on other nanostructures as well as for encapsulation of other nanoparticles of bioactive compounds) depends on physical properties of these fluids. At concentrations of 25% or higher, these liquids have high viscosity, thus, affecting their processing feasibility. This work attempts to study self-assembly of folic acid and folates at low to moderate concentrations and understand the changes in structure among low, moderate, and high concentrations of folic acid, as well as the impact of pH and ions on the structure and properties of the folic acid assemblies.

Experimental

Folic acid was acquired from CDH, New Delhi. An aqueous solution containing 5 wt % folic acid was prepared using purified water. (All concentrations in this study reported are in weight/weight basis.) One molar NaOH solution was added dropwise to this solution till the solution turned liquid crystalline (visually) while ensuring that the pH was less than 7.5. Solutions of other concentrations can be similarly made. Other alkaline solutions such as LiOH, KOH, and NH_4OH can also be used instead of NaOH.

In general, it was observed that folic acid did not dissolve in water as is, even at 0.1% concentration, by weight. It dissolved only on addition of NaOH or other bases of monovalent ions. Table 1 presents some data about the dissolution of folic acid in water. In each case, a folic acid solution was made by addition of folic acid to water. The solutions are not clear even after extended stirring. The second row of the table provides the gram moles of folic acid in 10 mL solution. The third row shows the pH of this solution.

To this solution, previously prepared 1N NaOH solution was added dropwise with constant stirring. pH was measured with each drop being added. The solution began to turn perlescent. At concentrations of 5% or higher, perlescent streaks of the solution were visible during stirring. At this point, no more NaOH was added. The fourth row of the table presents the final volume of NaOH solution added. The fifth row presents the moles of NaOH added. The sixth row shows the final pH.

At the state of dissolution, there is an equivalency in the gram moles of folic acid and NaOH. These experiments show that folic acid molecules dissolve in water only in their ionic state, when they are completely ionized from their acid forms. The pH of the dissolved ordered state is about 6.5. We also find that when the pH goes higher than 7.5, the basic solution causes hydrolysis of the folic acid, and the liquid crystalline solution turns into a dirty brown solution that does not show liquid crystalline behavior.

Similar solutions were made with LiOH and KOH as well. In each case, at the state of dissolution, folic acid solution had a pH around 6.5, and there was an approximate equivalency between the gmol of basic molecule required to dissolve an equal gmol amount of folic acid.

Microscopy Studies

Even at low concentrations (1% folic acid), one can see the formation of a perlescent solution that is often a sign of liquid crystallinity. Optical microscope images of the solution are presented in Figure 2 for 1, 5, and 10% solutions of folic acid in water with approximately equimolar amount of sodium hydroxide. A few drops of the solution at a given concentration were coated on a glass slide using a coating bar. This was placed under an optical microscope. The solution was viewed under an Olympus optical microscope (Model IX71S8F-3). The scale of these pictures is marked by a 200-μm bar at the bottom right corner of each figure.

The images show formation of aggregates even in 1% folic acid solutions. These aggregates seem to form continuous and extensive branched structures even at folic acid concentration of 5%. Concentration solutions (10%) show large domains of structured material. Although these optical images do not tell us much about the nature of organization, they do point out that a solution that seems homogeneous to the naked eye is, at a microscopic scale, structured and that the nature of the structure changes with concentration at the micron scale.

Figure 3 shows scanning electron microscope (SEM) images of a folic acid liquid crystalline solution. The sample was made by coating 20% folic acid solution on a substrate and letting it dry. Two snapshots from the same sample are shown later. One can see well-developed structures. The figure on the left shows a bimodal distribution of structures. Some are small-dust-like particles that are less than a micron. Others are plate-like structures that are about 5-μm wide and 10- to 50-μm long and that seem to have layers. It is possible that these are signs of nucleation of liquid

Table 1. Formulation of Folic Acid Solutions

Folic acid concentration (wt %)	1	5	10	15	20
gmol folic acid in 10 mL solution	0.00023	0.001136	0.0023	0.0034	0.0045
Initial pH	4.34	3.76	3.62	3.47	3.44
Volume of NaOH (mL)	0.25	1	2.5	3	4.5
gmol of NaOH	0.00025	0.001	0.0025	0.003	.0045
pH	6.2	6.6	6.5	6.47	6.44

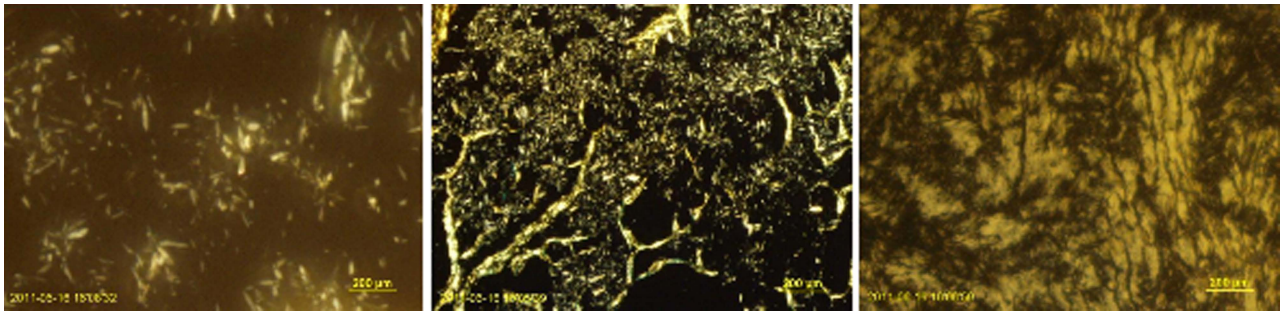

Figure 2. Optical microscope images of 1, 5, and 10% folic acid.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

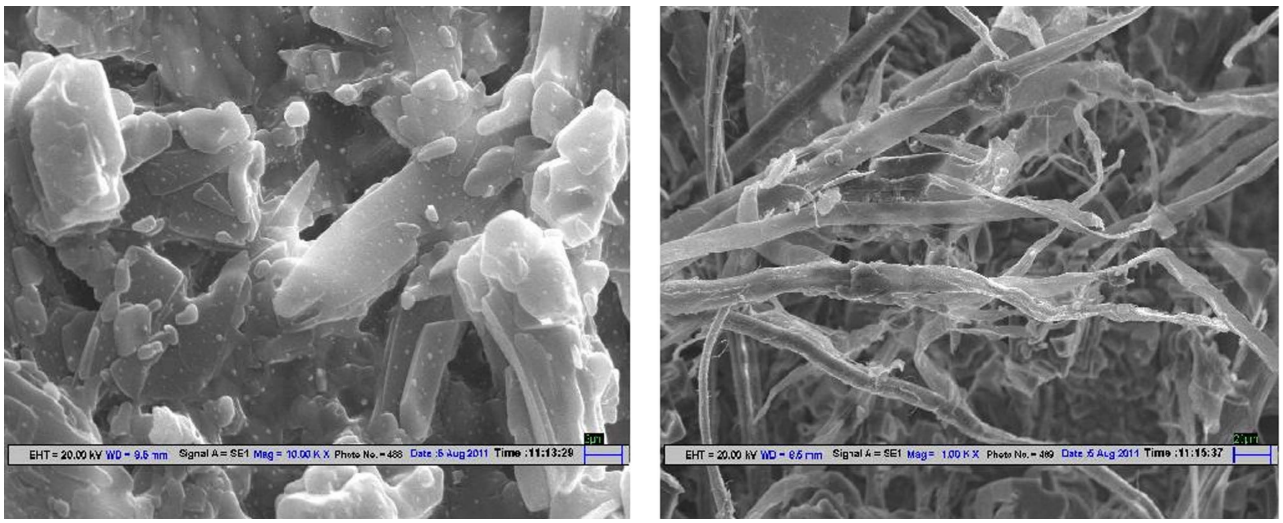

Figure 3. SEM images of a folic acid liquid crystalline solution—the figure on the left has a bar that is 2 μm and the one on the right has a bar that is 20 μm.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

crystalline structures. The figure on the right is another snapshot from the same sample and shows long-ribbon- and wire-like structures that are 2- to 10-μm wide and over 200-μm long. The sample suggests that during the process of drying, multiple structures have been formed with a distribution of shapes and sizes.

Tunneling electron microscope (TEM) images in Figure 4 show long-worm-like structures in 20% folic acid solutions. These structures are about 50-nm wide with a distribution of lengths. Some are over 200-μm long, thus, having aspect ratios that are 1:1000 or more.

X-Ray Diffraction Studies

Traditionally, a peak at about 3.3 Å was considered a signature of chromonics self-assembly, based on the hypothesis that $\pi-\pi$ interactions are fundamental to the chromonics assembly, though in the past decade, studies have shown that the assembly of chromonics is driven by van der Waals interactions rather than any specific $\pi-\pi$ interactions. The molecules assemble with their aromatic complexes facing each other, because the energy is optimized in the configuration. The spacing between the aromatic rings depends on the functional groups on the rings—interactions between functional groups around the ring can significantly change the spacing. Figures 5a,b show us the X-ray diffraction (XRD) profiles for a range of concentrations of folic acid. Table 2 shows us the *d*-spacing values of the peaks. Together, they help us understand the changes in XRD profile from which we could assess the structure of the ordered solution.

Thus, we see XRD peaks between ~3.0 and 4.2 Å that could possibly be attributed to spacing between rings. The XRD studies show a peak even at concentrations as low as 0.1% folic acid by weight. Up to about 0.5% concentration, there is a peak at about 3.9 Å and no other peak. Between

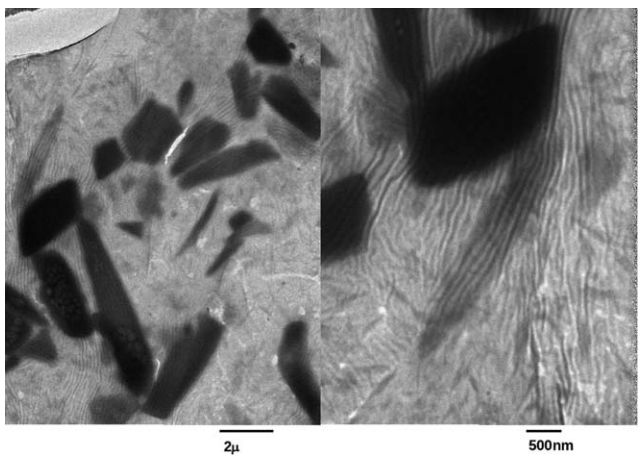

Figure 4. TEM images of 20% folic acid solution.

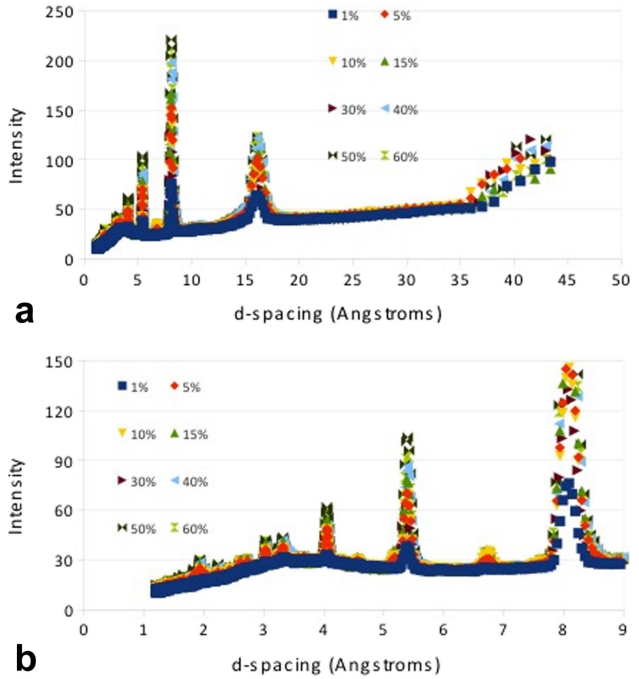

Figure 5. (a) XRD spectra of folic acid. (b) X-ray diffraction spectra of folic acid with an expanded *x*-axis to clearly show the peaks at about 3 Å.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

0.5 and 1%, the peak seems to shift to 4.0 Å. In addition, we see peaks at about *d*-spacings of 5.4, 8.1, 16.2 Å and a peak at a spacing of about 45 Å. At about 5%, the 4.04 Å peak disappears, and we see peaks at 3.03 and 3.3 Å that can be attributed to the spacing between the aromatic rings. As the concentration increases, the folic acid molecules reconfigure themselves to a structure that is perhaps more favorable at these concentrations. The two peaks point to difference in spacing between the two distinct aromatic complexes that are part of the folic acid molecule. It is also significant that these two peaks may change their intensities; however, they are present for folic acid solutions at all concentrations we studied—from 5 to 60%. The peaks at 5.4, 8.1, 16.2, and 45 Å persist at all concentrations higher than 0.5%. In fact, these are the strongest peaks of the XRD profile.

The presence of these peaks suggests that the structure of the folic acid structure does not change too much internally. Based on the structure of the folic acid molecule, it is hypothesized that the 5.4, 8.1, and 16.2 Å point to internal structure of the folic acid assembly. The large *d*-spacing peak is perhaps the broad spacing between the stacks. There are subtle changes within the peak profiles. Smaller peaks appear or disappear within ranges of concentrations, suggesting changes in phase. Based on these changes, this observation points to a phase—albeit an ordered phase—at very low concentrations where the XRD peaks suggest small clusters with little order between clusters (*d*-spacings of 3.9 is the only periodicity of this phase).

A second phase is observed between 0.5% and perhaps up to 1–5% where there is greater internal order in the structures (presence of 5.4, 8.1, and 16.2 Å). A third phase is observed between 5 and 15–30%, where along with the 5.4, 8.1, and 16.2 Å a large *d*-spacing peak is observed around 50 Å. In this concentration range, a peak is also observed at 6.7 Å, which is not observed at lower or higher concentrations. A fourth phase is observed between 15–30 and 50–60%, where the 6.7 Å peak has disappeared and the large *d*-spacing peak has shrunk closer to 45 Å. Finally, the data suggest another phase at concentrations higher than 60%. At 60% concentration, the solution is already paste like. This study has no data at higher concentrations, as the solution becomes viscous and difficult to formulate easily. The peaks at 16.4 Å is somewhat larger at this concentration. The large *d*-spacing peak perhaps exists but is higher than 50 Å—this study was not able to access peaks larger than 50 Å.

These results are significantly different from the results of Gottarelli.[15,16] These investigators present models of the self-assembled structures by analyzing the spacing between molecules and between stacks as deduced from XRD analysis. They further described the changes in these structures with changes in concentration by analyzing the shifts in such spacing. In addition to showing chirality of these self-assembled structures based on asymmetry of the molecule itself. Lokesh and Suryaprakash[22] have also analyzed this system showing that chirality occurs at higher concentrations of folate ions. SEM images further showed facets of such stacking. Based on this understanding, these studies speculated on possible configurations of the stacks with respect to each other. Unlike those results, this study sees more than two distinct peaks. In addition, the peaks do not change continuously. Rather, this study observes that the peaks are largely the same for similar phases, pointing to the change in phase as well as the significance of the internal assembly structure.

XRD studies were also done on folic acid solutions formulated with KOH and LiOH, as described earlier. The peak profiles of the folic acid solution at the given concentration are the same irrespective of whether the alkaline solution is NaOH, KOH, or LiOH.

Table 2. Analysis of XRD Spectra of Folic Acid Solutions at Increasing Concentrations

Concentration of Folic Acid (wt %)	Peak Positions (*d*-Spacing in Å)	Columns
0.1	3.9	
0.3	3.9	
0.5	4.04, 5.45, 8.22, 16.7, 43.05	No other peaks
1	4.04, 5.34, 8.08, 16.12, 42.38, 49.04	No other peaks
5	3.03, 3.32, 4.05, 5.39, 6.74, 8.08, 16.2, 48.1	Few low *d*-spacing peaks
10	3.03, 3.32, 4.05, 5.4, 6.73, 8.08, 16.17, 49.44	
15	3.02, 3.32, 4.05, 5.39, 6.72, 8.08, 16.25, 55.9	
30	3.03, 3.32, 4.05, 5.4, 6.74, 8.08, 16.07, 40.38	6.7 and larger peaks more intense
40	3.03, 3.31, 4.05, 5.4, 8.08, 16.08, 42.1	Numerous low *d*-spacing peaks
50	3.03, 3.31, 4.05, 5.40, 8.08, 16.09, 43.82	Numerous low *d*-spacing peaks
60	3.02, 3.31, 4.05, 5.39, 8.08, 16.14	Few low *d*-spacing peaks

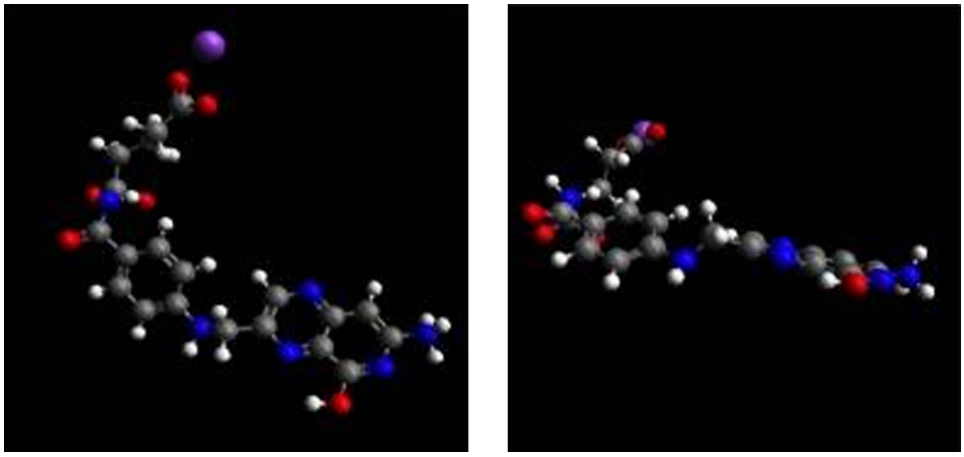

Figure 6. Configuration of foliate ion based on semiempirical calculations (two snapshots of the same structure).

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Semiempirical Calculations

This study, as well as past work, has posited that chromonics molecules form self-assembled solutions owing to their enthalpic interactions. However, it is also observed that numerous chromonics molecules (such as NMI from past research and folic acid from this work) form such assembled structures in solutions only when they are at a pH of 6–7. In both these examples, the molecules do not dissolve in water in their native state, which is a diacid state (where the solution with the undissolved settled solute) has a pH of between 3 and 5. This study has shown that enough base (equal to the gram moles of chromonics) has to be added for dissolution into a perlescent solution. Perhaps some semiempirical calculations can provide some reasons for such behavior.

The open source semiempirical quantum mechanics simulator Avogadro[23] was used to analyze folic acid molecules using MOPAC[24] with AM1[25–28] parameters. MMFF forcefields were used. The simulator was used to understand the single molecule (neutral or its ionized form), its charge distribution, and its conformations. This MOPAC (AM1) method was also used to do some preliminary studies of binding of these molecules or stacks at room temperature.

These calculations present that the molecule is neutral at pH up to 4. At solution conditions with $pH > 4$, the COOH groups lose a proton each. The dipole moment of the neutral molecule (at $pH < 4$) is 6.53 D, whereas that of the ion (at $pH > 4$) is 47.8 D. This prediction is consistent with observations in which the perlescent solution is not seen to form at low pH when the folate molecule is in its neutral state; only at pH close to 7 when the folate is in its doubly ionized state. The calculations suggest that the significantly increased dipole moment associated with change to an ionic structure facilitate much stronger interactions between the molecules, thus, facilitating liquid structure formation. We are, thus, interested primarily in the ionic state of the molecule.

Semiempirical studies using Avogadro are, at best, approximations that can provide us with trends, because the molecules are analyzed in vacuum or the presence of implicit solvents (vis-a-vis control of pH). The numerical values of the dipole moments should not be taken as accurate—however, they do point to significant change in the electronic structure as pH changes, and possible change in molecular structure and, hence, assembled structure of the molecules. The semiempirical calculations by Avogadro suggest a curled (Figure 6) folate ion structure at this pH. The figure shows two views of the molecule per semiempirical studies pointing to the curl as well as the twist in the structure. The twist is significant in which it suggests that the stack may have a chiral structure. However, given that these were calculated using implicit solvent, and this needs to be reassessed.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations were performed on folate ions using MD Darshan software,[29] a Graphics Processor Unit based MD software built on OpenMM platform[30] using CUDA.[31] It allows for fast simulations of relatively large systems with charges. Ionized folate ions with sodium counterions were simulated in an NPT ensemble (constant number of molecules, constant pressure, constant temperature ensemble) using Ewald summation. This set of simulations included 10 folate ions and 700 water molecules. The partial charges on these ions was calculated using Gasteiger Charge Distribution method.[32,33] In addition, formal charges were allotted to the ions. The simulations were run for 3000 ps at room temperature and pressure, though energy profiles approached their asymptotic values at 200–300 ps.

Throughout these simulations, we see that neither the folic acid nor the folate ions are in a curled state. The semiempirical calculations derived structures based on implicit solvents are inaccurate.

Figure 7a presents the results of the simulation using folate ions at 2000 ps. In the figure, for clarity of the structure of the folate ions, the water molecules have been hidden—thus, the apparent "empty" space is water. Unlike the semiempirical simulations with implicit water, the folate ions in this simulation show neither a curl nor a twist—in fact, the structure is almost planar. The two perspectives of the same snapshot shows stacking of the folate complexes with a high degree of order in a way that the aromatic rings face each other. This snapshot shows two stacks formed next to each other within the unit cell. Visually, there seems to be a high degree of order in the spacing between the molecules as well as the orientation.

Figure 7b presents the results of simulation using folic acid in water. The folic acid molecule, unlike the folate ion, has a greater out-of-plan twist between the two aromatic groups. There is no counter-ion and the COOH groups have not lost

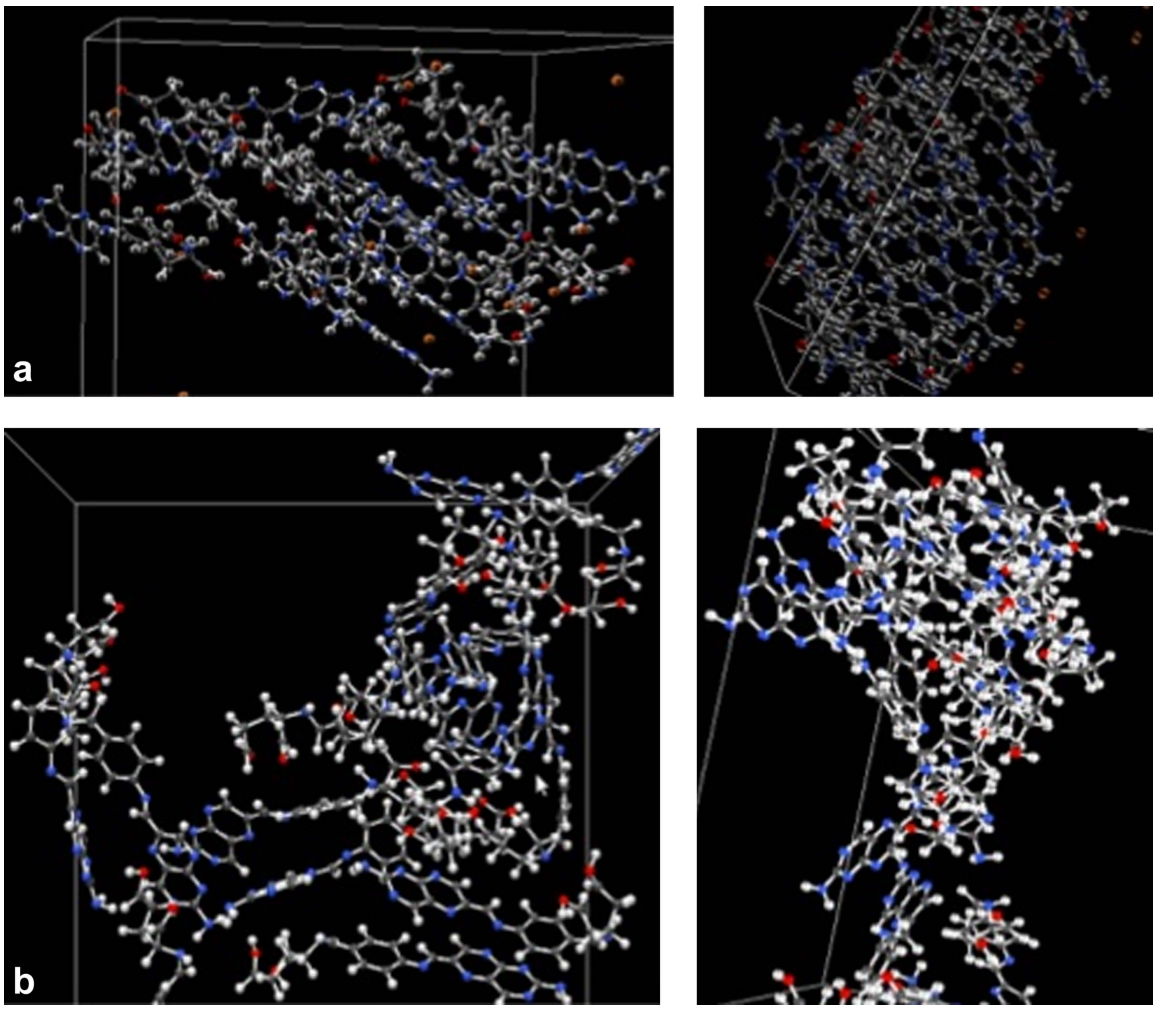

Figure 7. (a) Two views of assembled folate ions showing the configuration of the ions in the aqueous environment as well as the nature of order; (b) two views of assembled folic acid molecules showing the configuration in aqueous environment as well as the nature of order.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

their protons. Notice that the molecules have aggregated. However, there is little order among the molecules with little visible orientation order among the aromatic groups. This suggests a phenomenon where the folic acid ions may form some kind of suspended particles or precipitate.

Figure 8 presents the radial distribution functions (RDFs) of the foliate ions and the folic acid molecules, each in an aqueous environment at ~25% concentration. The RDF describes the distribution of nitrogens on the aromatic ring around a similar nitrogen atom on another aromatic ring. For the folate ions, RDF shows a peak at about 3.5 Å, corresponding to the aromatic group spacing presented by XRD. There are other peaks—suggesting spacing at other distances—however, they are much smaller and are at ~7

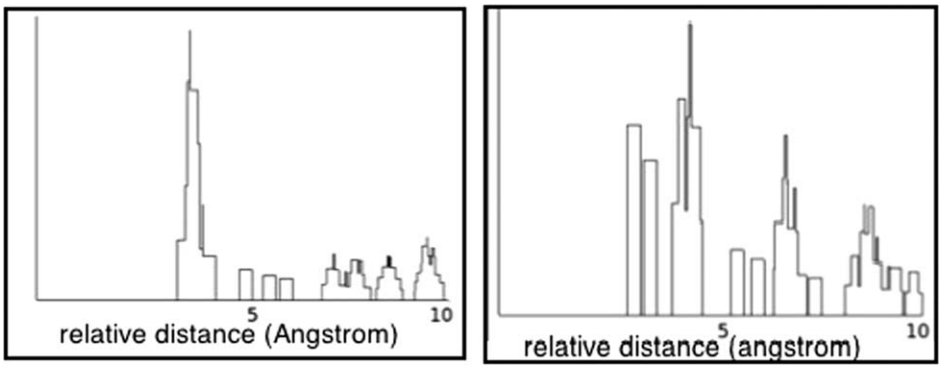

Figure 8. RDF.

(a) RDF of folate ions in their assembled state in aqueous environment. (b) RDF of folic acid molecules.

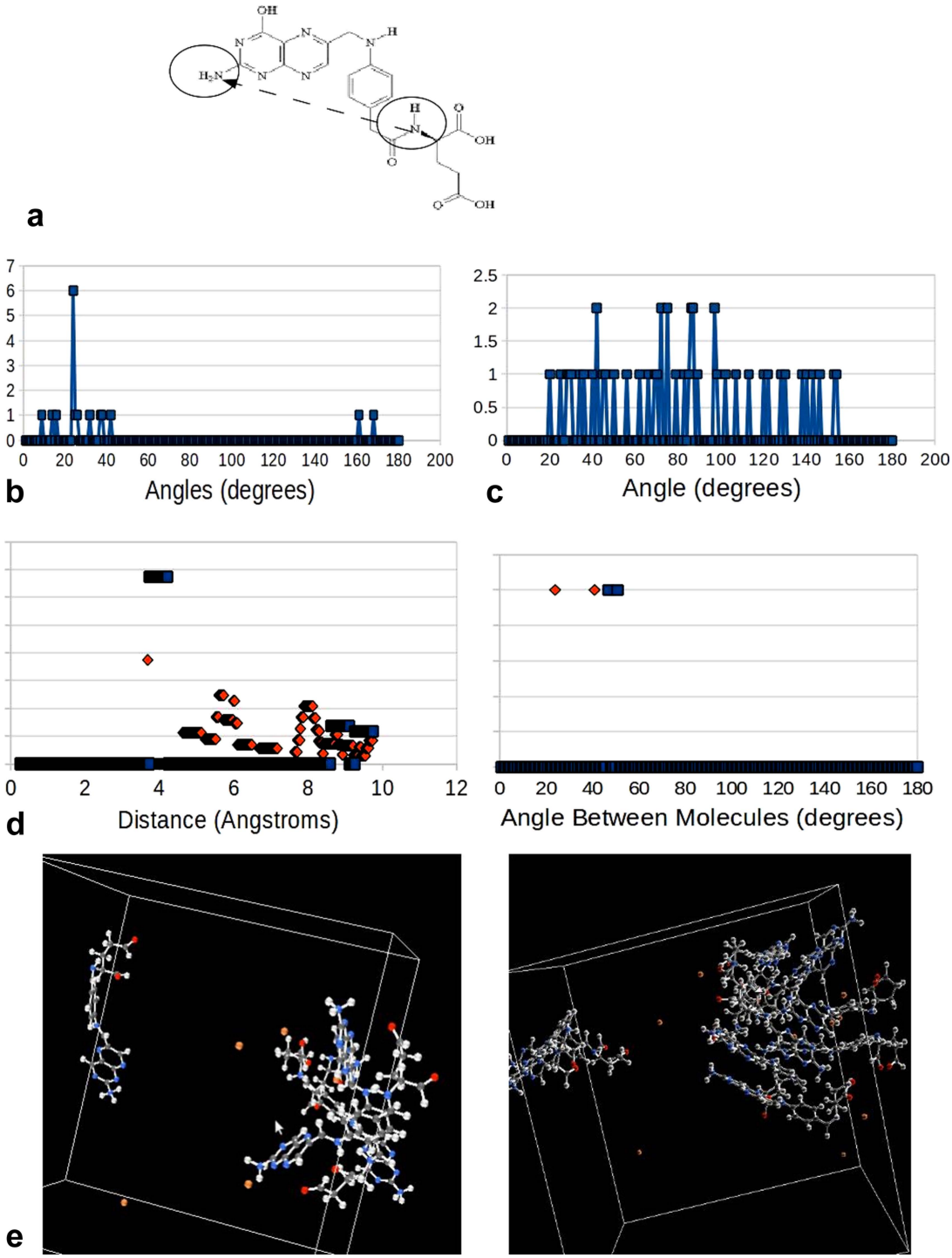

Figure 9. (a–c) Orientation correlation of foliate assemblies and folic acid assemblies.

(a) Defining the vector for orientation analysis; (b) frequency of occurrence angles between folate ions in the assembled structure; (c) frequency of occurrence of angles between folic acid molecules in the assembled structure. (d) RDF (left image) and the angle correlation (right image) of 5% (square markers) and 10% (diamond markers) foliate ions in their assembled states in aqueous environments. (e) Snapshots of foliate ion assemblies at 5% (left image) and 10% (right image) concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 10 Å suggesting that these are second and third neighbors. The presence of few other minor peaks suggests a high degree of order. In addition, there are peaks at about 5 and 8 Å. Based on the snapshot of the configuration shown in Figure 7, there are two stacks of folate ions adjacent to each other, and the distance between the π-rings in the two stacks is about 8 Å. The RDF of folate configuration is consistent with the XRD data, pointing to the peaks at 3.5, 5.5, and 8 Å.

For folic acid molecules, the RDF in Figure 8b presents a smaller peak at 3.5 Å and numerous other peaks of similar intensities suggesting a much broader distribution of distances between aromatic rings and little order.

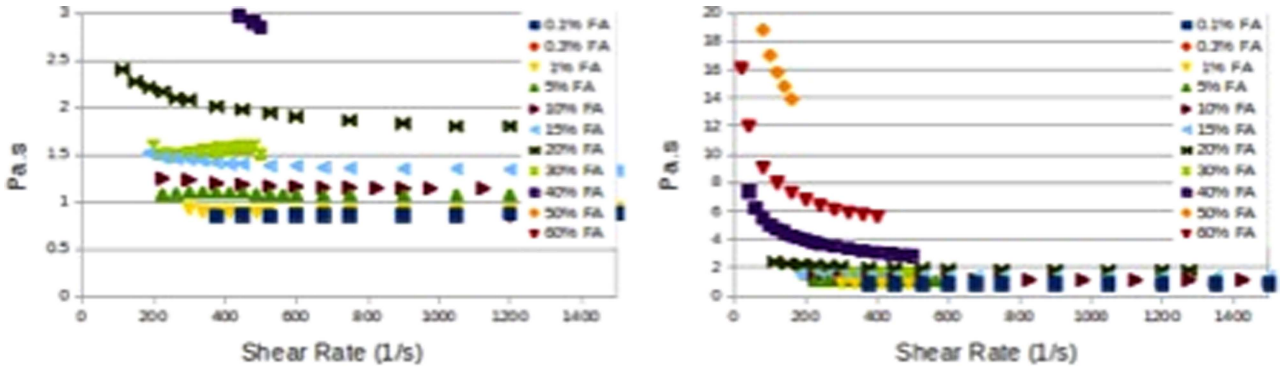

Figure 10. Rheological data of folic acid at different concentrations.

The two frames describe the same data at different scales of the *y*-axis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The configurations in the simulations are also analyzed to assess correlations in orientation. Figures 9b,c show the frequency of occurrence of the angle between any two folate ions or folic acid molecules. That is, a vector is defined by the position of the nitrogen atom to which the acid groups are connected with respect to the nitrogen atom dangling off the aromatic ring (see Figure 9a). The angle between the vectors of any two molecules helps us assess the relative orientations of the configuration. Figure 9b shows the relative orientation of molecules in a folate configuration. The *x*-axis is the angle between two molecules as defined earlier, and the *y*-axis shows the frequency of occurrence. Clearly, there is a predominant angle of orientation statistically validating the snapshot of the configuration presented in Figure 7. Also significant is that there is no occurrence where the molecules are oriented perpendicular to each other (i.e., angles close to 90°). Thus, in the range of these studies, tetramer structures were not found.

Figure 9c shows the relative orientation of molecules in a folic acid configuration. There is a broader distribution of angles with which the molecules orient themselves with each other. The RDF and the angle correlation studies suggest that the folic acid molecules configure such that $\pi-\pi$ interactions are possible; however, that is not a dominantly preferred position to the extent of folate ions. In addition, the folic acid molecules seem to have a broad range of orientation, whereas the folate ions seem to reside in a sharply focused narrow range of orientations. It could be concluded that the clusters of folic acid molecules are aggregates, perhaps precipitating aggregates with some order, whereas the folate ions show a highly ordered structure in their assembly.

Running simulations at lower concentrations of foliate ions show that the 3.5 Å peak persists (Figure 9d), though the other peaks shift somewhat. At 5% concentrations in these simulations (the square markers), the 3.5 Å peak is the dominant peak, whereas the other peaks are nonexistent or much smaller. At 10% concentrations of foliate ions (the diamond markers), there are other peaks (but at slightly shifted positions compared to 25%). In all these cases, the orientation of the molecules continues to be highly correlated. The 5% foliate ions (square markers) are oriented at about 40–60° with respect to each other, whereas the 10% ions (like the 25% ions) are closer to 20°. Clearly, there is an increase in order of the molecules with increasing concentration. Although the exact concentration of the simulations does not correlate with experimental studies, the trends hold.

In addition, at 5% concentration, a single stack of ions is observed (Figure 9e), whereas at 10% concentration, two stacks are observed where the molecules within each stack is oriented in the same way, though the orientations and positional alignment between stacks seems to be limited. At 25% concentration (Figure 7), the two stacks are much better aligned. This interaction between stacks should reflect a change in rheological properties of the solution as well.

Rheological Studies

Anton Paar Rheolab QC was used to measure rheological behavior of these solutions. Rheological studies validate the structured nature of folic acid solutions and the changes in structure at increasing concentrations. Figure 10 shows the rheological behavior of the self-assembled fluid with increasing concentration—all at room temperature. The response of the solution at very low concentrations is water like. At concentrations of 5–10% folic acid, we see signs of a structured liquid that are dependent on the rate of shear. The shear thinning is suggestive of an associative structure that breaks up at high shear rates. With increasing concentration, the apparent viscosity increases, whereas the rheological behavior is shear thinning. However, on increasing the concentration from 20 to 30%, there is a drop in the apparent viscosity. This is probably a sign of change in structure—perhaps at 30%, the liquid crystal stacks are more aligned, and, hence, the apparent viscosity is lower. Subsequently, at 60%, the apparent viscosity drops from the 50% folic acid concentration. Rheological evidence pointing to changes in structure at 5–10% concentration, at about 30–40% concentration, and at about 60% concentrations, which are consistent with XRD data.

The rheological analysis is significant beyond suggesting changes in phases that are consistent with XRD. Application of folic acid as a liquid crystalline material requires that the material exist in an ordered phase. Past work by Gottarelli and coworkers had pointed to its ordered state at concentrations of about 30% and higher. At these concentrations, the material exists as a paste with high viscosity and, thus, is difficult to process in forms of coatings or in formulating with other materials. This study is significant in its presenting that even at lower concentrations, the material has liquid has an ordered structure (though it may not be chiral) and has viscosities that are lower and, thus, can flow and be processed more easily. These results are key to leveraging the liquid crystalline behavior or the ordered structure of the system into applications of interest.

Conclusions

Past articles have suggested that folic acid shows assembled structures only higher than 25%. However, this study, through concurrence of XRD, microscopy, rheological data, and semi-empirical calculations, has clearly shown ordered assembly at low concentrations, as low as 0.1% folic acid by weight. In addition, this study pointed to the mechanism of formation of ordered structure in solution and the role of the univalent cation. It helped us understand the phases of aqueous solutions of folic acid, their transitions as well as possible molecular conformations and structure that leads to these phases.

Given past studies showing chromonics application in drug delivery, this study is valuable in showing clearly that folate solutions even at very low concentrations present ordered structures. Given that the ordered structures are known to be necessary for drug encapsulation in chromonics systems, this suggests that folic acid may be a feasible drug carrier. By presenting the conditions under which it shows ordered phase, it helps build the design space in which folic acid can be used as a drug carrier. It shows that the structure of the ordered configuration is different (at least at these concentrations) from the structures suggested by earlier studies, which would impact mechanisms of encapsulation as well as loading efficiencies. In defining the physical properties of folic acid under these conditions, it helps define processing conditions for its potential use.

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