Impact of Salts on Self Assembly of Folates

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Abstract- *This study characterizes the stability of folate self-assembly and the changes in their ordered structure and their properties with addition of various univalent cations and univalent and multivalent anions. Univalent cations play a key role in ionization and solubilisation of folates. The study shows that there is little impact in the choice of cation on the solubilisation of folic acid. Only at higher concentrations, the cations affect the structure of the folate assemblies and the rheology of the liquid crystalline solution. The choice of anions, however, makes a significant difference to the structure – it can change and even disrupt the order of the liquid crystalline structure. X-ray diffraction (XRD) is used to show that the behaviour of crystalline structure of folic acid with different concentrations of salts. Rheological studies are used to show existence of changes in their structure.*

Keywords- Folic acid; Chromonic; π-π Interactions; Self Assembly

Research highlights

- ➢ *Salts at higher concentration and their cation sizes are responsible for affecting the self assembly.*
- ➢ *XRD studies of these salts show that cations, anions also affects the ordered structure of folate.*
- ➢ *The sizes of ions also play the key role in disruption of ordered structure.*
- ➢ *Rheological data of these salts shows that cations, anions also affect the ordered structure of folate.*

Abbreviations

FA, Folic Acid
Na_2SO_3, Sodium Sulfite
NaBr, Sodium Bromide
LiCl, Lithium Chloride
KCL, Potassium Chloride
NaCl, Sodium Chloride
NaOH, Sodium Hydroxide
NH_4OH, Ammonium Hydroxide
LiOH, Lithium Hydroxide
KOH, Potassium Hydroxide
XRD, X-Ray Diffraction
CDH, Central Drug House
Wt. %, Weight Percent
Wt/wt, Weight by Weight

I. INTRODUCTION

One of the most important and vital process in chemical industries is Continuous stirred tank reactor (CSTR). CSTR is a highly nonlinear system and its parameters affect its complex dynamic severely. Due to nesting and deactivation and Chromonics are water-soluble molecules that contain complexes of planar aromatic rings with hydrophilic groups at their periphery. Example of chromonic molecules includes some dyes and drugs (for example methylene blue, methyl orange and disodium cromoglycate) [1-4]. These hydrophilic functional groups interact with water to help solubilise the chromonics molecules. In this solution, these molecules are stacked one above the other [5-8]. Chromonic molecules self-assemble even at low concentration by forming liquid crystalline solutions [9, 10]. The interaction which is responsible for formation of self-assembled stacks is enthalpic rather than entropic [11, 12]. Interaction of the aromatic rings influences the structure of the stacks [13]. We can possibly include guest molecule such as anticancer drugs, proteins or small molecule drugs between these stacks. Hence, chromonic materials are of greater interest as carriers in drug delivery [14-17]. Unlike micelles, the self-assembly of chromonic molecules do not show Krafft temperature and Critical micelles concentration [18-23]. Column and rod like structures are observed in the liquid crystalline solution formed by these chromonic molecules [23].

Folic acid is one of the compounds representing the class of chromonics molecules (Figure 1). The structure of

folic acid includes two sets of aromatic rings with hydrophilic functional groups at their periphery [9, 10, 20, 21]. Folate molecules show liquid crystalline behavior due to the presence of the pterin nucleus in their structure which associates with other pterin molecule [19, 22]. We have already studied folic acid assembly, in the presence of NaOH [9, 10]. Clearly sodium cations have a role to play in the assembly. There is an interest in understanding how these cations affect the liquid crystal structure, and further, in exploring how anions affect their structure.

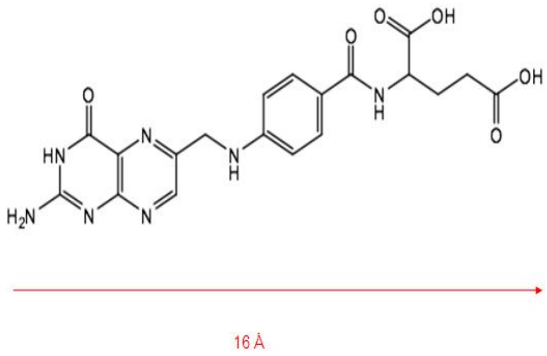

Figure 1. Molecular structure of folic acid.

Raghavan et al [23] has reported a significant change in rheology of solution at higher concentration of salts. We are interested in how order can be controlled and how properties of the solution changes with changing anions, cations at varying concentrations. In this study, we use X-ray diffraction (XRD), to understand the changing structure of assembly with changing ions and with changing concentration. The effect of salts like sodium chloride, sodium bromide, sodium sulphite, potassium chloride and lithium chloride were considered to investigate their effects on folate self-assembly. Rheological studies were done to understand the impact of changing structure on the properties of these fluids. In general, viscosity of these fluids affects the ability to process them in mixing and in drug encapsulation.

II. MATERIALS & METHODS

2.1 Materials

Folic acid (purity approximately 96%) and all salts (sodium chloride, sodium bromide, sodium sulphite, potassium chloride and lithium chloride) were purchased from Central Drug House (CDH), New Delhi. While, Sodium Hydroxide, Lithium hydroxide and Potassium hydroxide are purchased from Sigma Aldrich.

2.2 Preparation of Folate Self Assembly

An aqueous stock solution of 5 wt. % of folic acid was prepared using deionized water. (All the concentrations in this study are reported in weight/weight basis). One molar NaOH solution was added drop wise to this solution to make it liquid crystalline at the same time making sure that the pH was less than 7.5 during mixing. The pH affects the liquid crystallinity and stability of the solution [9, 10, 25]. The liquid crystalline solution turns into dirty brown solution when pH crosses 7.5. Similarly, 10 wt. % of folic acid solution can be made. The liquid crystalline solution was also made with other bases such as LiOH, KOH and NH_4OH instead of NaOH.

Folic acid does not dissolve in water even at low concentration, by weight. It dissolved only on addition of NaOH or other bases of monovalent ions. To this solution, previously prepared 1N NaOH solution was added drop wise with constant stirring. pH was measured with each drop being added. The solution began to turn perlescent. At this point, no more NaOH was added and solution shows the liquid crystalline behavior.

Folic acid dissolves in water only when, there is an equivalency in the gram moles of folic acid and NaOH. Thus, 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 state folic acid solution was found to be about 6.5. We also find that when the pH goes higher than 8, the alkaline solution causes hydrolysis of the folic acid and the liquid crystalline solution turns into a transparent brown color solution that does not show liquid crystalline behavior.

All salts (sodium chloride, lithium chloride, sodium bromide, potassium chloride, sodium sulfite) were dissolved in folate solution (5 wt. % and 10 wt. %) at different concentrations (0.75, 1.5 & 2.25 wt. %) and (1.5, 3 & 4.5 wt. %) separately.

2.3 Characterization of Folate Self Assembly

2.3.1 X-Ray Diffraction (XRD)

Changes occur in folate self-assembly due to the impact of salts were observed using X-Ray Diffraction (Rigaku Mini Flex 600 with Mini Flex PDXL software). Thin film of the folate suspension was prepared and analyzed at scanning speed of 4.0 deg. /min with X-ray beam at 40 kV, 15 mA.

2.3.2 Rheology

Different solutions of folate with salts and changes occur in their structure were observed using Anton Paar

Germany GmbH with RHEOPLUS/32 V3.62 SN 21006425-33056 software (MCR302 SN81193479). Prepared solutions was analyzed from Shear rate 10 to 500 (1/s) with CP 50-1; d=0.1 mm.

III. RESULTS &DISCUSSION

3.1 X-Ray Diffraction Studies

The changes occuring in the folate assembly are analysed using XRD. Figure 2 shows the X-ray diffraction (XRD) profiles of 5 wt. % and 10 wt. % solutions of folate. We focus in this range of concentrations because they are of interest to possible drug delivery applications. At much higher concentrations, the viscosity increases significantly making processing more difficult. At lower concentrations, inclusion of guests becomes difficult. Figure 2 shows that the peak intensities increase slightly with increasing concentration, pointing to a slightly more ordered structure. However, there are no new peaks formed. The peaks approximately at diffraction angles of 5º, 10º, 16º, 21º and 27º correspond to d-spacing values of 16.2 Å, 8.1 Å, 5.4 Å, 4 Å and 3.3 Å respectively. As reported in past studies, 3.3Å d-spacing corresponds to interaction between aromatic rings of folate ions (Figures 3 & 4) [9, 10]. Interaction between the functional groups around the aromatic rings of folate assembly can change the d-spacing as shown in figure 3. It is hypothesized that these peaks correspond to the structure of folic acid assembly.

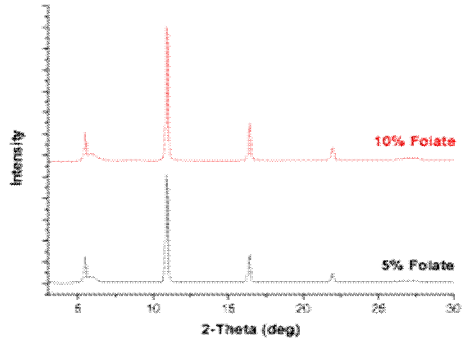

Figure 2. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate solutions.

3.1.1 Structural Analysis of Folate Self Assembly

The length of the single monomer of folic acid is around 16 Å as shown in figure 1. Earlier work has shown that assembly is based on aromatic ring interactions. Thus, we can assume that the folate structures are stacks of pterin rings that allow for aromatic ring interactions. Past studies have shown that multiple in-plane configurations of pterin ring based molecules are possible in an assembled stack [19]. ChemBio 3D Ultra (Version 12.0) software is used to identify possible structures of folate assembly which corresponds to these peaks. Figure 3 shows the possible in-plane structures of dimer, trimer and tetramer geometry of folate self-assembly. The structural arrangements of dimer shows in-plane dimension of 25 Å. A trimer shows in-plane dimensions of 27 Å, 4 Å & 8 Å. A tetramer shows in-plane dimensions of 33 Å, 4 Å & 6 Å respectively. These configurations are only approximate configurations and provide an estimate of the dimensions of the in-plane configurations. These in-planar configurations of folate form stacks owing to aromatic ring interactions [9, 10] as schematically shown in figure 4.

Comparison of these approximate dimensions of the Chem Draw structures to XRD peak positions suggests that these folate ions perhaps stack in trimer or tetramer configurations reflecting the 4 Å, 6 Å & 8 Å positions.

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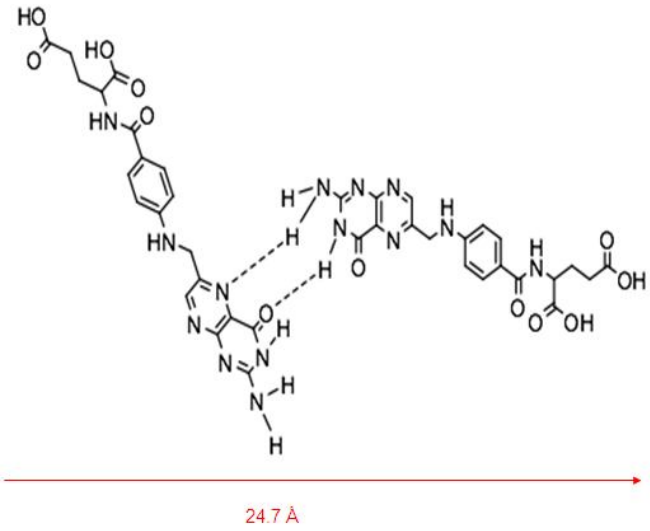

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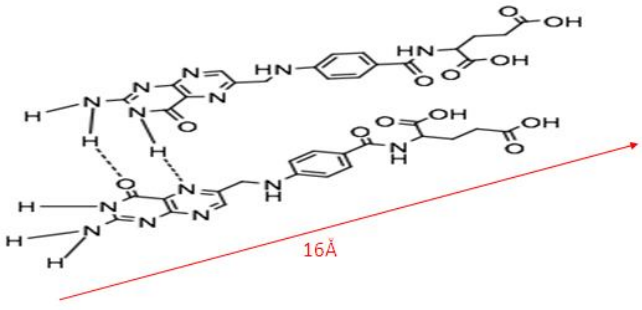

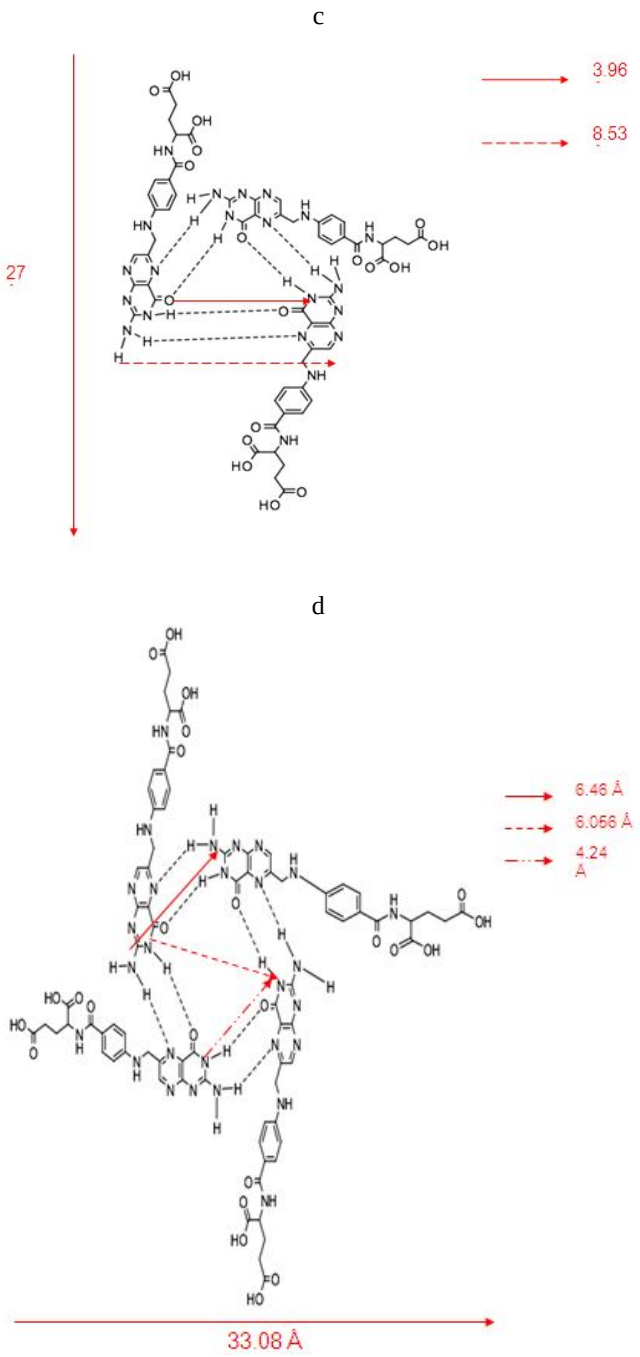

Figure 3. (a and b) Dimer, (c) Trimer and (d) Tetramer structutes of folate

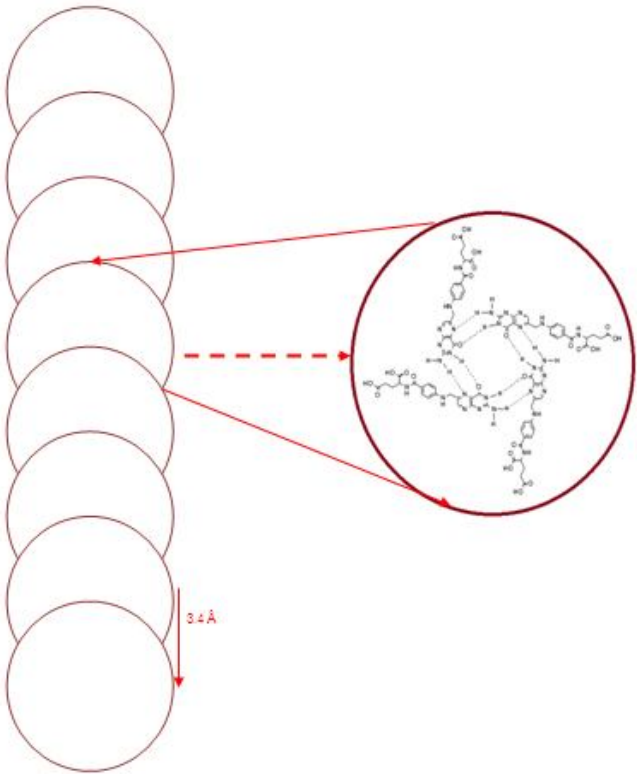

Figure 4. Self-assembled structure of folate.

3.1.2 Impact of Base Alkaline solutions

Folic acid is insufficiently soluble between pH 1 and 6. Poe et al [24] has shown that folic acid has pKa values of – 1.5, 0.2, 2.35, 2.40, 5.71 and 8.38. The two COOH groups are ionized separately; both are ionized between pH of 1 – 6. The amide group is ionized above 8.0 pH. This confirms our hypothesis based on earlier studies that folic acid dissolves only after both COOH groups are fully ionized [9, 10]. This is consistent with the study by Poe et al. that the liquid crystalline structure of folic acid is visible between pH of 6 to 7.5. Above 8.0, the solution turns brown. Thus, folic acid forms liquid crystalline solutions in water with equivalent moles of base.

The folate solution was made using different alkaline solutions (NaOH, LiOH and KOH). There was no visual difference in the liquid crystalline behavior using different bases. The peaks positions for liquid crystalline solutions made with different alkaline solutions do not change. However in LiOH solutions, the peak intensities are higher than NaOH and KOH (both of which behave in a similar manner). This implies that more ordered structure of folate self-assembly is formed when LiOH is used as the base. This is possibly due to the sizes of cations in these bases, since cation sizes of Li^+, Na^+ and K^+ are 0.9 Å, 1.16 Å and 1.52 Å respectively.\

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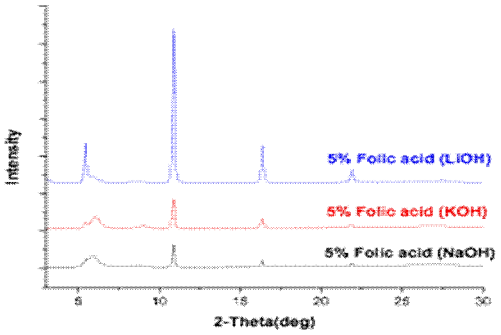

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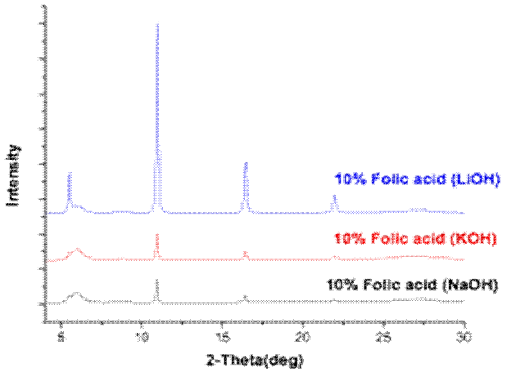

Figure 5. X-ray diffraction spectra of (a) 5 wt. %folate with alkaline solutions (b) 10 wt.% folate with alkaline solutions.

3.1.3 Impact of Salts on Folate Self Assembly

Cations seem to affect the extent of order of folate ions. Exploring the impact of ions of the assembled structure even further, X-Ray Diffraction studies of folate with changing salt concentrations were also performed. In the present study, we have used sodium chloride, sodium bromide, sodium sulphite, potassium chloride and lithium chloride allowing for a comparison of the impact of different anions and cations on the assembled structure.

3.1.4 Impact of NaCl on Folate Self Assembly

Figure 6 shows the X-ray diffraction (XRD) pattern of 5 wt. % and 10 wt. % of folate solutions with sodium chloride salt at different concentrations (0.75, 1.5 & 2.25 wt. %) and (1.5, 3 & 4.5 wt. %) respectively. The peaks at diffraction angle of approximately 5º, 10º, 16º, 21º and 27º correspond to d-spacing values of 16.2 Å, 8.1 Å, 5.4 Å, 4 Å and 3.3 Å respectively, which is same as observed in pure folate self-assembly. The key difference is the broadening of the peak at diffraction angle between 26º and 27º. The intensities at diffraction angles 26º and 27º decreases with increases the concentration of sodium chloride from 0.75 to 2.25 in 5 wt. % and 1.5 to 4.5 in 10 wt %.

At 5 wt. % folate concentration with NaCl (0.75 %), the peak intensity at diffraction angle of 5º is higher than other concentrations of NaCl. Perhaps the stacks that are formed are disrupted at higher concentrations of salt. At 4.5 % NaCl concentration with 10 wt. % folate, peak at diffraction angle of 10º decreases, while it increases from 1.5 to 3 % of NaCl. Thus, ordered structure disrupted at high concentration of sodium chloride though there may be changes in their geometric structure depending on the nature of packing. Hence, peaks suggested that sodium chloride plays the role in the formation of ordered structure of folate.

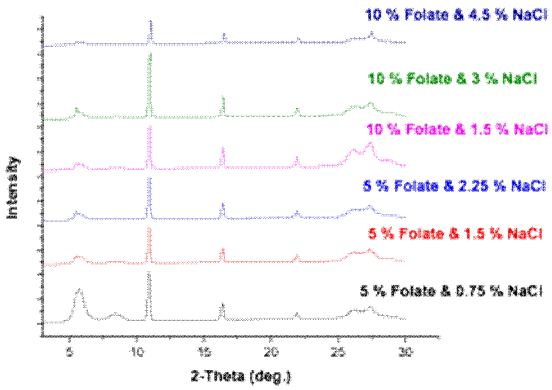

Figure 6. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with NaCl salt.

3.1.5 Impact of LiCl on Folate Self Assembly

X-ray diffraction (XRD) profile of folate assembly (5 wt. % and 10 wt. %) with lithium chloride salt at different concentrations (0.75, 1.5 & 2.25 wt. %) and (1.5, 3 & 4.5 wt. %) respectively are shown in figure 7. Lithium chloride shows similar behaviour that were earlier observed in sodium chloride except at diffraction angle of 10º. At 5 wt. % folate concentration with LiCl (2.25 %), peak at diffraction angle of 10º decreases, while it increases from 0.75 to 1.5 % of LiCl. There are no significant changes in XRD spectra of sodium chloride and lithium chloride though there may be changes in their geometric structure depending on the nature of packing.

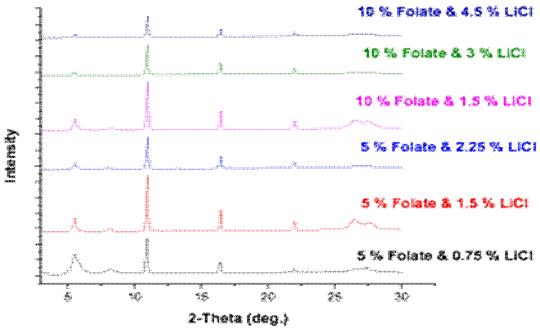

Figure 7. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with LiCl salt.

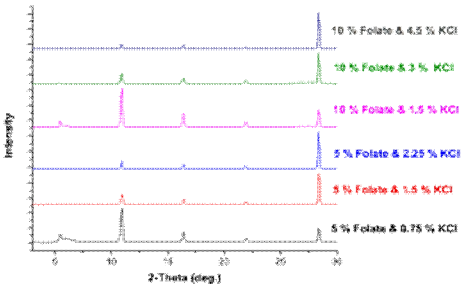

Figure 8. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with KCl salt.

3.1.6 Impact of KCl on Folate Self Assembly

X-ray diffraction (XRD) profile of folate at 5 wt. % and 10 wt. % mixed with potassium chloride at different concentrations (0.75, 1.5 & 2.25 wt. %) and (1.5, 3 & 4.5 wt. %) respectively (figure 8). The peaks positions are same as observed in pure folate solution except the peak at diffraction angle of 27º shifts to 28.37º. The peak shows higher intensity as compared to pure folate, or folates formulated with sodium chloride and lithium chloride. The peak intensity at diffraction angle of 28.37º increases with increases the salt concentration in 5 and 10 wt. %. The d-spacing value at diffraction angle of 28.37º is 3.14 Å. This peak corresponds to the aromatic rings of folate stacks. It is possible that a large cation in the vicinity of the delocalized aromatic rings results in the decrease in spacing between the stacked aromatic rings. Similar to sodium chloride and lithium chloride, peaks intensities at diffraction angles of 5º, 10º, 16º and 21º decreases with increases the concentration of potassium chloride; in fact the decrease of intensities is much more marked. Perhaps the stacks that are formed are disrupted at higher concentrations of salt.

Peaks positions of folates with different salts stay largely the same compared to pure folate solution. However in addition to that changes in peaks intensities are marked. The electronegativity of lithium chloride is higher than sodium chloride and potassium chloride {LiCl (0.98) > NaCl (0.93) > KCl (0.82)}. Hence, lithium cation shows higher charge density than sodium cation and potassium cation; as ionic radius of lithium cation is small. Possibly the folate stacks are affected due to the charge density of these salts. XRD profiles suggest that cation sizes of these salts affected the packing geometries of folate. Thus, univalent cations size plays a key role in the formation of ordered structure.

To compare the effect of salts on folate self-assembly, XRD study was carried out at 5 wt. % of folate with different salts at concentration of 0.75 wt. %. Figure 9 shows the X-ray diffraction (XRD) profile of folate with different salts. The peaks position of 5 wt. % folate shows no change on addition of salts. However, folate with KCl salt shows high peak intensities at all positions, whereas lower intensities were observed with LiCl salt. This implies that ordered structure of folate self-assembly is not largely disrupted by KCl salt as compared to LiCl. This is possibly due to the charge density of these cations. The charge density of these cations is in the sequence of $Li^+ > Na^+ > K^+$ (Hofmeister series) [23, 27]. Lithium cation shows higher charge density than sodium cation and potassium cation; as ionic radius of lithium cation is small as compared to other two ions, therefore more disruption in folate ordered structure was observed.

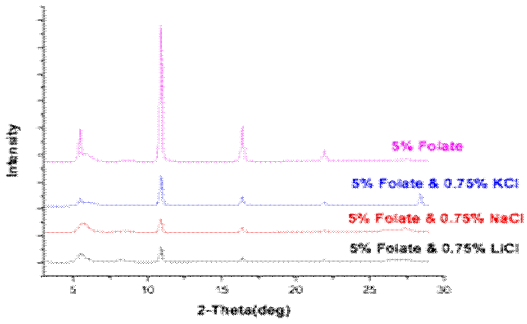

Figure 9. X-ray diffraction spectra of 5 wt. % folate with different salts.

3.1.7 Impact of Na_2SO_3 and NaBr on Folate Self Assembly

Figure 10 shows the X-ray diffraction (XRD) profiles of 5 wt. % and 10 wt. % of folate with different concentrations of Na_2SO_3 and NaBr salts. The peaks approximately at diffraction angles of 5º, 10º, 16º and 27º corresponds to d-spacing values of 16.2 Å, 8.1 Å, 5.4 Å and 3.3 Å respectively.

These peaks are significant to folate self assembly and dimensions of folate stacks. These peaks are observed only at low concentration of Na_2SO_3 (0.75 %); while they disappear at higher concentration (about 1.5 to 4.45) of Na_2SO_3 salt. Thus, ordered structure of folate is disrupted at higher concentrations of this salt. On addition of sodium bromide in folate solution, a new peak is observed at d-spacing 6 Å (diffraction angle 14.7º). The intensity of this peak increases as sodium bromide concentration is increased from 0.75% to 2.25% at 5 wt. % folate. The intensities of all other peaks that were earlier observed in folate assemblies significantly decrease with increasing NaBr concentration.

On comparing the effect of NaBr and NaCl salt, it was observed that the peak at diffraction angle of 14.7º (present in NaBr) was disappeared in the case of NaCl. At 5 & 10 wt. % folate concentration with NaCl, peak intensity at diffraction angle of 10º decreases with increases the concentration of NaCl. However at 5 wt. % of folate with NaBr, peak intensity at diffraction angle of 10º decreases with increasing concentration of NaBr; in fact the decrease in intensities was much more significant at 1.5 & 2.25 %. At 5 wt. % folate concentration with NaCl (0.75 %), the peak intensity at diffraction angle of 5º is higher than other concentrations of NaCl and NaBr. At 10 wt. % of folate, peak intensity at diffraction angle of 16º increases with increasing concentrations of NaBr, while in NaCl (both 5 & 10 wt. % of folate) the intensities decreases with increasing the concentration of NaCl. Moreover at 5 wt. % folate concentration with NaBr (1.5 %), peak at diffraction angle of 16º is lower than other concentrations of NaBr (0.75 & 2.25 %) and NaCl (1.5 %).

Earlier we point out that cations are responsible for the disruption of ordered structure. Over here, sodium chloride and sodium bromide contain same cation but with different anion also show the changes in the XRD profile. Therefore, we can say that anions also play the role in formation of ordered structure. From XRD studies, we have shown self-assembled structure of folate is disrupted with the impact of univalent cations and univalent and multivalent anions of salts. Thus, both anions, cations and their sizes are responsible for the disruption of folate assembly.

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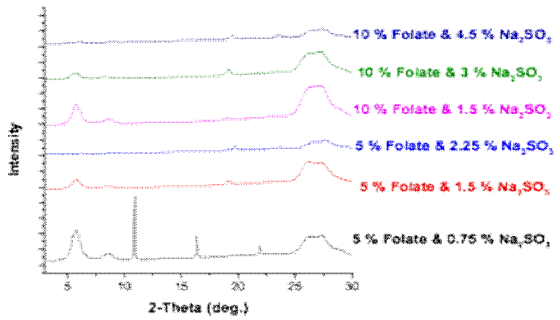

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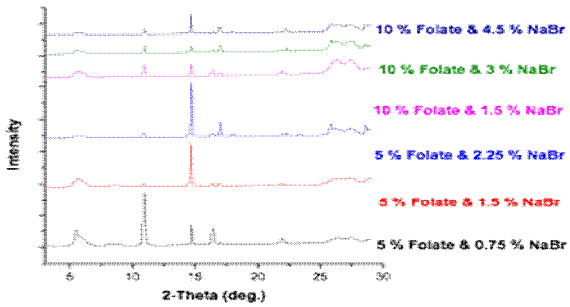


Figure 10.X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with (a) NaBr (b) Na_2SO_3.

Table 1.Analysis of XRD spectra of 5 wt. % and 10 wt. % folate with NaBr and Na_2SO_3salts.

Folate (% by weight)	Salts	Folate: Salts (wt./ wt. basis)	Major peaks	π-π stacking
5	NaBr	5:0.75	16.07, 8.05, 6.01, 5.39, 4.05	3.3
		5:1.5	16.07, 8.07, 6.03, 5.39, 4.05	3.3
		5:2.25	16.11, 8.07, 6.03, 5.39, 4.05	3.3
	Na_2SO_3	10:1.5	16.38, 8.10, 5.41, 4.08	3.2
		10:3	15.17, 4.63	3.2
		10:4.5	14.45, 4.49	3.2
10	NaBr	5:0.75	16.11, 8.07, 6.02, 5.39, 4.05	3.3
		5:1.5	16.11, 8.07, 6.03, 5.39, 4.05	3.3
		5:2.25	16.11, 8.07, 6.03, 5.39, 4.05	3.2
	Na_2SO_3	10:1.5	15.10, 4.63	3.4
		10:3	15.81, 4.62	3.4
		10:4.5	14.57, 4.50	3.3

3.2 Rheological Studies

A rheological study can be useful to predict the impact of guest ions on self-assembled structure of folate. In the present study, rheological studies were performed to measure the behavior of 5 wt. % and 10 wt. % of folates

without salts and with different salts — at all room temperature (figure 11). The apparent viscosity of 5 wt. % and 10 wt. % of folate are different, while 10 wt. % of folate shows the shear thinning behavior. As folate stacks are randomly arranged in the system, shear thinning is observed due to these stacks which aligned themselves in the direction of shear force. Therefore, apparent viscosity gradually decreases with increases the shear rate. The difference in apparent viscosities was observed with different folate solutions in presence of different salts. The variation of the apparent viscosities is due to the charge density of the cations. Cations possibly affect π-π interaction between the aromatic parts of the molecules and between the aggregates of folate stacks by inducing the changes in the hydrogen bonding. Past studies have shown that cation spatially fits into the cavities on the surfaces of the chromonic stacks [23, 26]. The sequence of the charge density of the cations are $Li^+ > Na^+ > K^+$ (Hofmeister series) [23, 27]. The charge density of Li^+ is higher than K^+ and therefore disruption of folate stacks are more in LiCl salt. Hence the apparent viscosity is higher in LiCl salt as compared to NaCl and KCl. However, the charge density of K^+ is comparetively lower than Na^+ and Li^+ ions and it is hypothesized that size of K^+ ions matches the cavities present on the surfaces of the folate stacks. This may enhance the stability of folate stacks and therefore the apparent viscosity is lower.

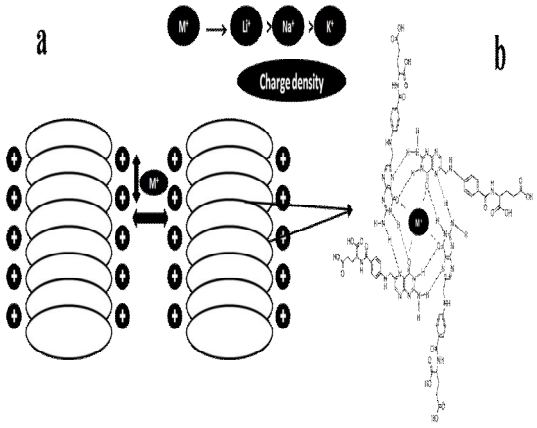
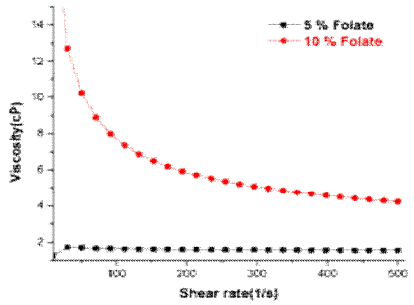


Figure 11. (a) Folate stacks disrupted by cations and (b) Tetramer structure contains the metal ions (M^+)

With increasing concentration of lithium chloride, the apparent viscosity increases and shear thinning behavior was observed. This is probably a signature of change in structure – perhaps folate stacks are more aligned at lower concentration which is disrupted at higher concentration of salt and hence viscosity is high. Similarly, rheological behavior of sodium chloride, potassium chloride and sodium bromide show shear thinning as shown in figure 12; while the apparent viscosities of these solutions varies with salts concentration. However in sodium sulphite, there is a drop in the apparent viscosity on increasing the salt concentration from 0.75 to 2.25 % with 5 & 10 wt. % respectively. Therefore, we can say that the structure of folate stacks disrupted at higher concentration of salt. Comparing the sodium chloride and sodium bromide, the apparent viscosities are significantly different and this suggested that chloride anion also plays the important role in the disruption of folate stacks.

The rheological data is consistent with the XRD profiles of salts. Raghavan et. al. [23] also pointed that the ordered structure of chromonic liquid crystals affected by the higher concentration of salts and size of the cations. This study is significantly suggesting that ordered structure and their properties are affected by varying the concentrations of various univalent cations and univalent and multivalent anions of salts.

a



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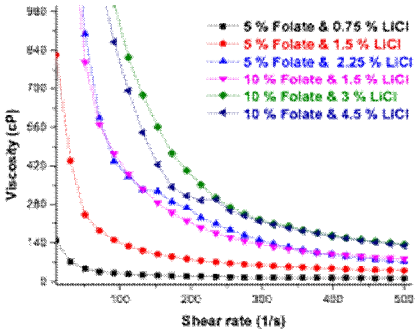

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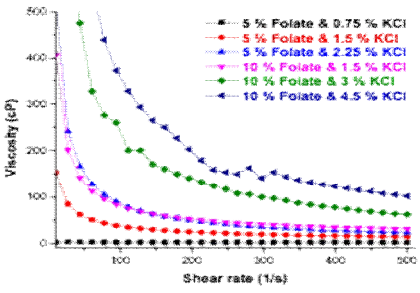

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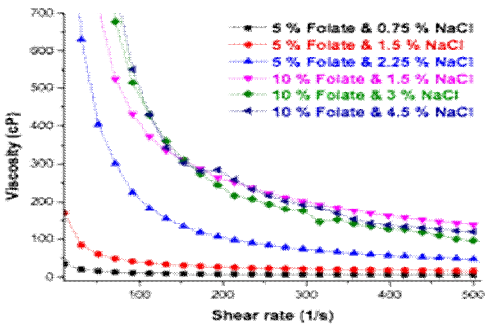

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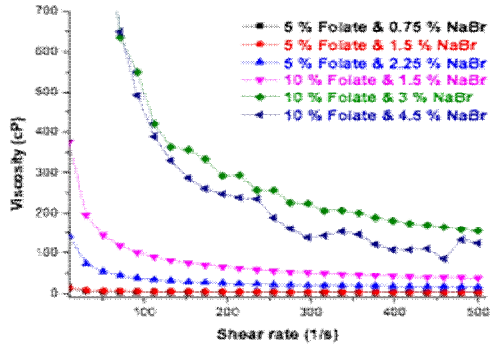

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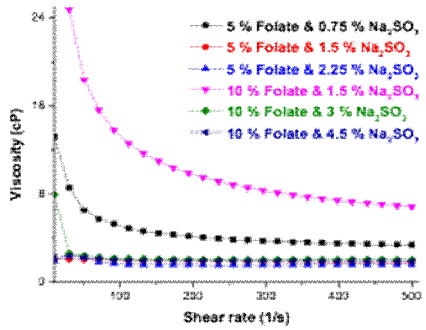

Figure 12. Rheological data of (a) 5 & 10 wt. % folate solutions. (b) 5 & 10 wt. % folate with LiCl salt. (c) 5 & 10 wt. % folate with NaCl salt. (d) 5 & 10 wt. % folate with KCl salt. (e) 5 & 10 wt. % folate with NaBr salt. (f) 5 & 10 wt. % folate with Na_2SO_3 salt.

IV. CONCLUSION

Past studies suggested that salts at higher concentration and their cation sizes are responsible for affecting the self assembly. However, In this study we have clearly shown that at different concentration of salts; both anions and cations disrupted the self assembled structure of folate through X-ray diffraction (XRD) and rheology. Moreover, the sizes of ions also play the key role in disruption of ordered structure. Multivalent anions such as sodium sulphite are totally disrupted the assembled structure at higher concentration of salt. From XRD studies and rheological data of these salts, we hypothesized that along with cations, anions also affects the ordered structure of folate.

Figure Captions:

Figure 1. Molecular structure of folic acid.
Figure 2. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate.
Figure 3. Dimer (a and b), (b) Trimer and (c) Tetramer structutes of folate
Figure 4. Self-assembled structure of folate.
Figure 5. X-ray diffraction spectra of (a) 5 wt. % folate with alkaline solutions (b) 10 wt. % folate with alkaline solutions.
Figure 6. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with NaCl salt.
Figure 7. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with LiCl salt.
Figure 8. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with KClsalt.
Figure 9. X-ray diffraction spectra of 5 wt. % folate with different salts.
Figure 10. X-ray diffraction spectra of 5 wt. % and 10 wt. % folate with (a) NaBr (b) Na_2SO_3.
Figure 11. (a) Folate stacks disrupted by cations and (b) Tetramer structure contains the metal ions (M^+).
Figure 12. Rheological data of (a) 5 & 10 wt. % folate solutions. (b) 5 & 10 wt. % folate with LiCl salt. (c) 5 & 10 wt. % folate with NaCl salt. (d) 5 & 10 wt. % folate with KCl salt. (e) 5 & 10 wt. % folate with NaBr salt. (f) 5 & 10 wt. % folate with Na_2SO_3 salt.

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REFERENCES

[1] Tanford C. The Hydrophobic Effect: Formation of Micelles and Biological Membranes. New York: Wiley, 1973; Chapter 1.

[2] Lydon J. New models for the mesophases of disodium cromoglycate. Mol Cryst Liq Cryst. 1980; 64:19–24.

[3] Lydon J. Chromonic liquid crystal phases. Curr Opin CollInt Sci. 1998; 3(5):458–466.

[4] Horowitz VR, Janowitz LA, Modic AL, Heiney PA, Collings PJ. Aggregation behavior and chromonic liquid crystal properties of an anionic monoazo dye. Physical Review E. 2005; 72:041710.

[5] Lydon J. Chromonic mesophases. Current Opinion in Colloid and Interface Science. 2004; 8:480–490.

[6] Kato T, Mizoshita N. Self-assembly and phase segregation in functional liquid crystals. Current Opinion in Solid State and Materials Science. 2002; 6:579–587.

[7] Dickinson AJ, LaRacuente ND, McKitterick CB, Collings PJ. Aggregate Structure and Free Energy Changes in Chromonic Liquid Crystals. Mol Cryst Liq Cryst. 2009; 509:751–762.

[8] Mohanty S. Chromonics: Reviewing a High-performance Self-assembling Structure. Indian Chem Eng. 2011; 53(2):84–94.

[9] Motkar G, Lonare M, Patil O, Mohanty S. Self-Assembly of Folic Acid in Aqueous Media. AIChE J. 2013; 59(4):1360–1368

[10] Misra R, Lonare M, Gupta R, Mohanty S.Engineering Chromonic Nanoparticles from Liquid-Crystalline Folate Solutions.Science of Advanced Materials J. 2014; Vol. 6, pp. 1–9.

[11] Mohanty S. Molecular simulation of self-assembly of hydrophilic functionalized aromatics in aqueous solutions. Mol Simul. 2006; 32(8):633–642.

[12] Mohanty S, Chou S-H, Brostrom M, Aguilera J. Predictive modeling of self-assembly of chromonics materials. Mol Simul. 2006; 32:1179–1185.

[13] Collings PJ, Dickinson AJ, Smith EC. Molecular aggregation and chromonic liquid crystals. Liquid Crystals. 2010; 37:701–710.

[14] Gupta R, Kalita P, Patil O, Mohanty S. An investigation of folic acid–protein association sites and the effect of this association on folic acid self-assembly. J Mol Model. (2015) 21:308

[15] Misra R, Katyal H, Mohanty S. Controlled release of folic acid through liquid-crystalline folate nanoparticles. Materials Science and Engineering C. 44 (2014) 352–361

[16] Gupta R, Mohanty S. Quantification of BSA in Presence of Folic Acid. International Journal of ChemTech Research, CODEN (USA): IJCRGG ISSN: 0974-4290 Vol.7, No.2, pp 800-803, 2014-2015

[17] Misra R, Mohanty S. Sustained release of methotrexate through liquid-crystalline folate Nanoparticles. J Mater Sci: Mater Med (2014) 25:2095–2109

[18] Prasad SK, Nair GG, Hegde G, Jayalakshmi V. Evidence of Wormlike Micellar Behavior in Chromonic Liquid Crystals: Rheological, X-ray, and Dielectric Studies. J Phys Chem B. 2007; 111(33):9741–9746.

[19] Kanie K, Nishii M, Yasuda T, Taki T, Ujiieb S, Kato T. Self-assembly of thermotropic liquid-crystalline folic acid derivatives: hydrogen-bonded complexes forming layers and columns. J Mater Chem. 2001; 11:2875–2886.

[20] Ciuchi F, Nicola GD, Franz H, Gottarelli G, Marisni P, Bassi MGP, Spada GP. Self-Recognition and Self-Assembly of Folic Acid Salts: Columnar Liquid Crystalline Polymorphism and the Column Growth Process. J Am Chem. 1994; 116:7064–7071.

[21] Bonazzi S, DeMorais MM, Gottarelli G, Mariani P, Spada GP. Self-Assembly and Liquid Crystal Formation of Folic Acid Salts. Angew Chem Int Ed Engl. 1993; 32(4):248–250.

[22] Kamikawa Y, Nishii M, Kato T. Self-Assembly of Folic Acid Derivatives: Induction of Supra molecular Chirality by Hierarchical Chiral Structures. Chem Eur J. 2004; 10:5942–5951.

[23] Kostko AF, Cipriano BH, Pinchuk OA, Ziserman L, Anisimov MA, Danino D, Raghavan SR. Salt effects on the phase behavior, structure and rheology of chromatic liquid crystals. J Phys Chem B. 2005; 109:19126–19133.

[24] Poe M. Acidic dissociation constants of folic acid, dihydrofolic acid, and methotrexate. J Biol Chem. 1977; 252(11): 3724-3728.

[25] Wu Z, Li X, Hou C, Qian Y. Solubility of Folic Acid in Water at pH Values between 0 and 7 at Temperatures (298.15, 303.15, and 313.15) K. J. Chem. Eng. Data. 2010; 55(9): 3958–3961

[26] Kato T. Self-Assembly of Phase-Segregated Liquid Crystal Structures. Science.2002;295:2414–2418

[27] Nucci N, Vanderkooi J. Effects of salts of the Hofmeister series on the hydrogen bond network of water. Journal of Molecular Liquids. 200