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Lyotropic chromonic liquid crystalline nature of berberine in ortho-phosphoric acid

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ABSTRACT

We report the results of our studies on the optical and thermal properties of the mixture of two non-mesogenic compounds viz., Berberine (BBE) and Orthophosphoric Acid (H_3PO_4). The mixture exhibits very interesting induced smectic phases, such as SmA, SmC* and SmE phases for different concentrations of BBE sequentially when the specimen is cooled from its isotropic phase. Different liquid crystalline phases observed in these mixtures were studied using DSC, X-ray and Optical microscopic techniques. The temperature variation of optical anisotropy has also been discussed. Helfrich potential and Elastic moduli have been estimated in the smectic phase using Helfrich model.

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KEYWORDS

Berberine;
Binary mixture;
Lyotropic chromonic;
Nematic;
Helfrich potential;
Mesomorphic phases.

INTRODUCTION

Thermotropic and Lyotropic liquid crystallline materials are possessing one or more mesophases between the isotropic liquid and the solid phase. The mesophase sequence, observed when the temperature is changed, is due to a sequential ordering of molecular arrangements. There is a special class of lyotropic liquid crystals, called lyotropic chromonic liquid crystals (LCLC). The LCLC family embraces a range of dyes, drugs, nucleic acids, antibiotics, carcinogens, and anti-cancer agents. The shape of molecules is then fundamental to determine the features of this sequence. The conventional lyotropic liquid crystals are based on amphiphilic rod-like molecules with polar heads and hydrophobic alkyl chain tails, also referred to as surfactants. LCLC molecules are believed to be plank-like rather than rod-like, rigid rather than flexible, aromatic rather than aliphatic. The material can achieve a nematic phase characterised by an orientational order of molecules. In the smectic phases, the order increases: besides the orientational order, the molecules have an arrangement in layered structure, in some cases with tilt (smectic C) or positional order within the layers (for instance in smectic B)[1-7].

In present study the material being strongly anisotropic, with optical birefringence properties, a common technique applied to detect the liquid crystalline behaviour in the polarized microscopy, the sample was sandwiched between the slide and cover slip, typically with thickness of about 20 to 30 microns. The temperature driven phase transitions are the most beautiful phenomena that can be observed, with sudden changes of colours and textures of different liquid crystalline phases observed and they have been verified from the results of DSC and X-ray studies. In light of the above investigations, an attempt has been made to understand the coupling between aggregate structure and the mesophase order in light of the observations regarding lyotropic chromonic liquid crystals[8], where-in, it has been observed that, the aggregates formed at low concentrations are not large enough to align, and, at larger concentrations, aggregate size increases into supramolecular assemblies. The exact size of the aggregates is concentration dependent, and birefringence only occurs when it is energetically favorable for the aggregates to orient themselves in a uniform direction, which requires them to be sufficiently large. Observation of lyotropic chromonic liquid crystalline nature in a mixture has been reported in this paper. Helfrich potential and elastic modulii have been estimated in the lamellar smectic phases using Helfrich model with approximation.

MATERIALS UNDER STUDY

Berberine

Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. It is found in such plants as Berberis. As a traditional medicine or dietary supplement, berberine has shown some activity against fungal infections, Candida albicans, yeast, parasites, and bacterial/viral infections[9,10]. Berberine seems to exert synergistic effects with fluconazole even in drug-resistant Candida albicans infections[11].

Berberine is considered to be an antibiotic[12,13]. When applied in vitro and in combination with methoxyhydnocarpin, an inhibitor of multidrug resistance pumps, berberine inhibits growth of Staphylococcus aureus[14] and Microcystis aeruginosa[15] a toxic cyanobacterium. Berberine is a component of some eye drop formulations. There is some evidence it is useful in the treatment of trachoma[16], and it has been a standard treatment for leishmaniasis[17]. Berberine lowers elevated blood total cholesterol, LDL cholesterol, triglycerides and atherogenic apolipoproteins[18,19].

Ortho phosphoric acid

Ortho phosphoric acid is used in dentistry and orthodontics as an etching solution, to clean and roughen the surfaces of teeth where dental appliances or fillings will be placed. It is also an ingredient in anti-nausea medications that also contain high levels of sugar. It is used for high-performance liquid chromatography and it is the ingredient that provides acidity to Coca-Cola and Pepsi sodas and even used as a pH adjuster in cosmetics and skin-care products[20].

The structural formulae of Berberine and Orthophosphoric acid are shown in Figures 1(a, b).

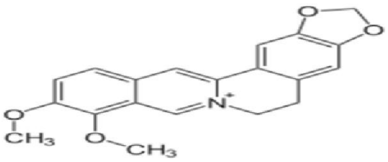

Figure 1(a) : Structural formula of the berberine (BBE) molecule

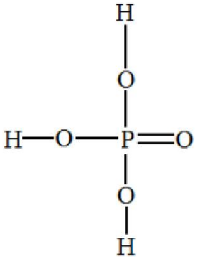

Figure 1(b) : Structural formula of the ortho-phosphoric acid (H_3PO_4) molecule

EXPERIMENTAL

Mixtures of twenty different concentrations of BBE in H_3PO_4 were prepared and they were mixed thoroughly. These mixtures were kept in desiccators for six hours. Samples were subjected to several cycles of heating, stirring and centrifuging to ensure homogeneity. Phase transition temperatures of these mixtures were measured with the help of a polarizing microscope in conjunction with a hot stage. The samples were sandwiched between the slide and cover slip and were sealed for microscopic observations. DSC thermograms were taken for mixtures of all concentrations using the Perkin-Elmer DSC II Instrument facility available at Raman Research Institute, Bangalore, India. The phase diagram was obtained by plotting the phase transition temperatures of the mixtures, which were determined by the DSC studies as a function of concentrations of BBE in H_3PO_4. The phase diagram (Figure 3) clearly indicates that the mesomorphism of the mixture is thermodynamically stable but only partially ordered phases and each mesophase is described by its own degree of order. The X-ray diffraction studies were done using Jeol X-ray diffractometer at various temperatures for different concentrations, which exhibit a smectic phase[21-23]. The density and refractive indices of the mixtures were measured at different temperatures employing the technique described in our earlier paper[24].

RESULTS AND DISCUSSION

Optical studies

The polymorphic smectic modifications and the corresponding isotropic to liquid crystalline phase transition temperatures for the mixture with 60% of BBE in H_3PO_4 are given below.

Iso-141°C, SmA-108 °C, SmC*-64.5 °C, SmE -40.2°C.

On cooling the specimen from its isotropic melt, the setting point is marked by the genesis of nucleation at several points which appear as minute bubbles initially, but which progressively grow radially and form a focal conic fan texture of smectic-A phase in which the molecules are arranged in layers and the texture is shown in the Figure 2(a). The SmA phase is unstable and then changes over to SmC* phase, which exhibits radial fringes on the fans of focal conic textures, which are the characteristics of chiral SmC* as shown in Figure 2(b). The molecular twist can be of the constituent molecules in the chiral smectic-C phase and they posses point symmetry in relation to their asymmetric centers, when they are packed in the form of layers, where the molecular long axes are tilted with respect to the layer planes and the phase has C_2 symmetry[25,26]. The stacking of the layer planes on the other results in the tilt possessing about normal to the layers creating macroscopic helical structures. On further cooling, this phase changes over to the crystalline SmE phase, which remains up to room temperature.

The partial phase diagram shown in Figure 3, illustrates that the mixture with concentrations from 10% to 85% of BBE exhibits a SmA, SmC* and SmE phases sequentially when the specimen is cooled from its isotropic melt. The mixture with concentrations from 10% to 20% of BBE does not exhibit SmC* phase but exhibits SmA and SmE phases only. The interesting feature of phase diagram of the present mixture is that it exhibits the unusual sequence of chiral SmC* phase in the mixture with concentrations ranging from 20% to 85% of BBE with the temperature range of 72°C to 123°C. The molecular structure of BBE is shown in Figure 1(a). It can be clearly seen from the molecular structure that it does not contain the alkyl chains. It might be the reason for the exhibition of interesting phase sequence by the mixture containing this compound which is departing from the conventional sequence of phases.

Observation of optical textures clearly shows that the mixture is exhibiting the nature of lyotroic chromonic liquid crystals[8]. Up to 10% concentration of BBE in H_3PO_4, the mixture is not showing any clear mesophase. The mixture exhibits only a birefringent region and it is difficult to associate the texture with any of liquid mesophases. The most remarkable feature of these BBE molecules is the tendency of their constituent parts to segregate in space with the creation of interfaces. Evidently the polymorphism seems to be entirely depending on the interfacial behaviour and this behaviour ultimately leads to the limiting of the polymorphism for homogeneous interfaces for higher concentrations of BBE molecule. It can be noticed that the phase transition temperatures observed in the present study are dif-

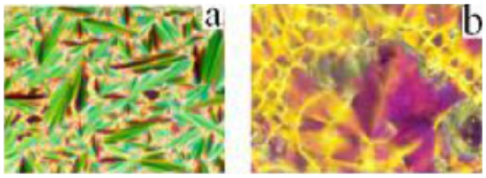

Figure 2 : Microphotographs obtained in between the crossed polars, (a) Focal conic fan shaped texture of SmA (Lamellar) phase (250X); (b) Chiral SmC* phase (180X)

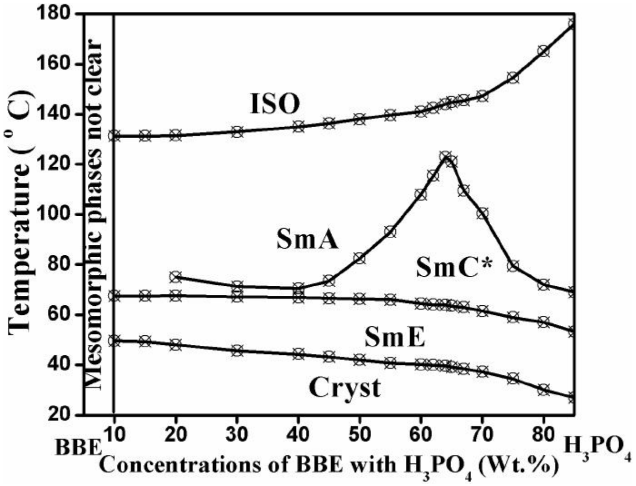

Figure 3 : Partial phase diagram for the mixture of BBE in H_3PO_4

ferent from the values observed in a similar type of study by Nagappa et al.[27] in which the mixture of a different compound with H_3PO_4 has been studied. Hence the interaction of H_3PO_4 looks to be different with different compounds.

X-ray studies

To understand the change in layer spacings in smectic-A and smectic-C* phases with respect to temperature, X-ray diffractometer traces were taken. The traces obtained for the mixture of 60% BBE in H_3PO_4 at different temperatures correspond to smectic-A and smectic-C* phases. It is observed that, as the temperature increases the layer spacing also increases in smectic-C* phase, but in smectic-A phase the layer spacing is almost constant[28-30] and these variations are as shown in Figure 4.

Optical anisotropy

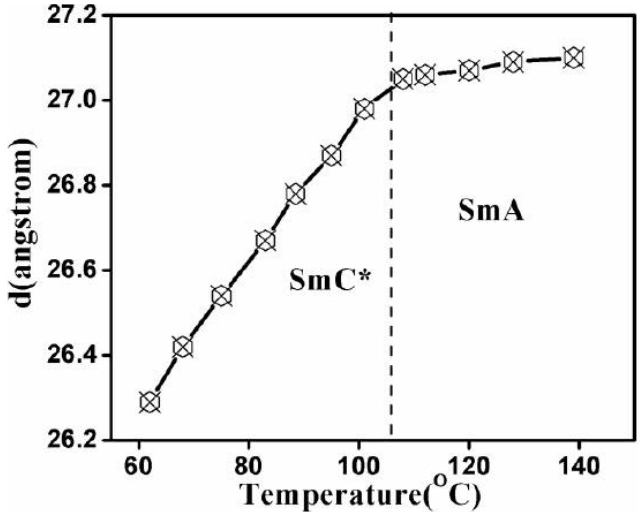

Figure 4 : Variation of layer spacing with temperature

Results of this investigation are further supported by the optical studies. The refractive indices for extraordinary ray (n_e) and ordinary ray (n_o) of the mixture were measured at different temperatures for different concentrations using Abbe Refractometer and precession Goniometer Spectrometer. The temperature variations of refractive indices for 60% of BBE in H_3PO_4 are as shown in Figure 5. The value of n_e is greater than n_o, indicating that the material is uniaxial positive. The values of electrical susceptibility for 60% of BBE in H_3PO_4 have been calculated using Neugebauer relation[31] at different temperatures. The temperature variations of electrical susceptibility for the mixture are as shown in Figure 6. From the figure, it can be observed that wherever there is an isotropic-liquid crystalline phase transition, the value of electrical susceptibility changes appreciably, which indicates that each change corresponds to various smectic modifications. Further, with increase in the concentration of BBE, the value of electrical susceptibility decreases with temperature, because the effective optical anisotropy associated with the molecules of BBE also decreases.

Helfrich potential and elastic modulus

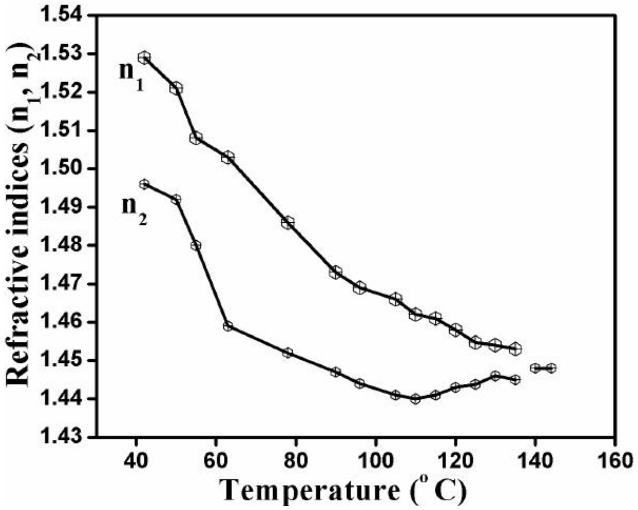

Figure 5 : Temperature variation of refractive indices for the mixture of 60% BBE in H_3PO_4

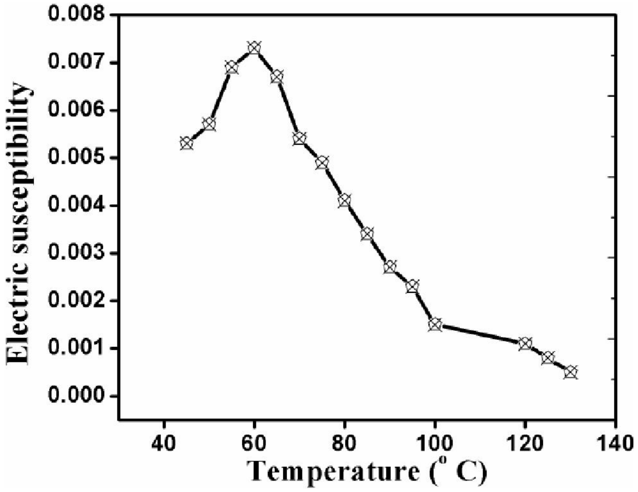

Figure 6 : Temperature variation of electric susceptibility for the mixture of 60% BBE in H_3PO_4

The free energy of steric inter-membrane interaction exists between undulating neighbouring membranes, when they are side by side in the multilayer systems[32]. The undulation modes in multilayer systems can be treated in terms of the de Gennes theory[33] of fluctuations in smectic phase, which invokes curvature elasticity and smectic compressibility. To estimate the Helfrich potential (V (ξ)), we consider the free energy per unit area,

$$V(\xi) = \beta \frac{(k_B T)^2}{k_0 \xi^2} \qquad (1)$$

where $\beta = 3\pi^2/128$, $(k_0/k_B T) = 0.75$ (The repulsive force between the membranes), k_0=bare bending constant, k_B is the Boltzman constant.

The V (ξ) of membrane varies with inverse square of the membrane spacing assumed that the local tilt of the membrane induced by undulations remains in effect well below π/2. ξ is mean membrane separation. Here it has been considered that ξ is equal to d[34], and its value is 26.53 Å.

Variations of Helfrich potential along with the concentrations of BBE are as shown in Figure 7 and it is very interesting to note that, the Helfrich potential value increases as the concentration of the BBE increases. This result invokes that in dilute region of the mixture, V (ξ) value is low. This is supported by the nature of variation exhibited by the elastic modulus.

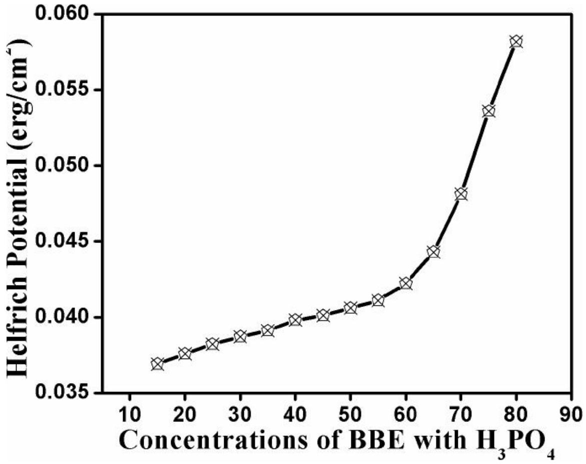

Figure 7 : Variation of Helfrich potential with concentration of BBE in H_3PO_4

The elastic modulus (K)[34] of smectic compressibility is calculated using the relation,

$$K = \frac{3\pi^2}{64} \frac{(k_B T)^2}{k_c d} \qquad (2)$$

where k_c is curvature elastic modulus.

The elastic modulus is also estimated for the mixture of different concentrations at various temperatures. The graph obtained by plotting the elastic modulus as a function of the concentration of BBE is presented in the Figure 8. From the graph it is observed that as the concentration of BBE decreases, the value of bulk modulus also decreases.

The reason for the small values of electrical susceptibility, bulk modulus and Helfrich potential in low concentrations is due to the lesser value of density in which the interaction of smectic layers with the neighbouring layers is very less.

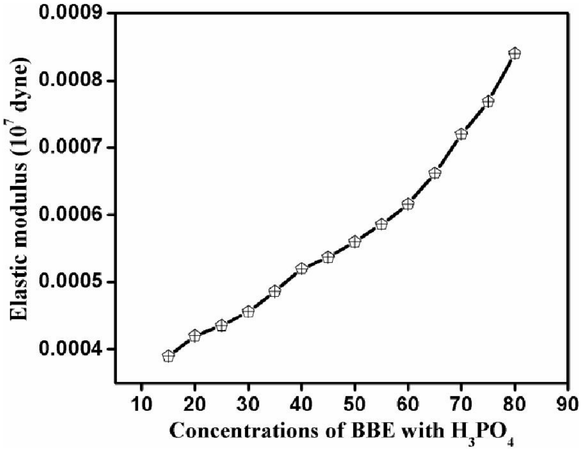

Figure 8 : Variation of elastic modulus with concentration of BBE in H_3PO_4

CONCLUSIONS

Microscopic investigation of the binary mixture of BBE and H_3PO_4 shows the existence of induced smectic phases, such as SmA, SmC* and SmE phases for different concentrations of BBE molecule. An observation from the various studies of this unconventional sequence clearly indicates that the mixture is exhibiting the lyotropic chromonic liquid crystalline nature. At lower concentrations of BBE in H_3PO_4, the aggregates are not aligned and they start aligning as the concentration increases at higher temperatures. The drastic changes in the values of density, refractive index and anisotropy of polarizability with temperature unambiguously correspond to smectic phase. The nature of temperature variation of layer spacing in SmC* and SmA phases look to be different as observed from X-ray diffraction studies.

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REFERENCES

[1] I.Kasianova, E.Kharatyian, A.Geivandov, S.Palto; Liq.Cryst., **37(11)**, 1439 **(2010)**.

[2] T.Felekis, L.Tziveleka, D.Tsiourvas, C.M.Paleos; Macromolecules, **38(5)**, 1705 **(2005)**.

[3] P.J.Collings, M.Hird; Introduction to Liquid Crystals, Taylor & Francis: Bristol, PA., **(1997)**.

[4] J.Lydon; Chromonics, In: Handbook of liquid crystals. Wiley-VCH, Weinheim, **2B**, 981 **(1998)**.

[5] S.Chandrasekhar; Liquid Crystals, Cambridge University Press, **(1992)**.

[6] G.Gray, J.Goodby; Smectic Liquid Crystals, Leonard Hill, Glasgow and London, **(1984)**.

[7] D.Demus, L.Richter; Texture of liquid crystals, VEB Deutscher verlag fur grundstoffindustrie, Leipzig, **(1978)**.

[8] Elizabeth Mills; http://www.swarthmore.edu/x32235.xml. Advisor: Peter collings, Thesis: Analysis of IR-806 Aggregation and Chromonic Liquid Crystal Properties, **(2011)**.

[9] T.C.Birdsall, G.S.Kelly; http://www.thorne.com/media/berberine.pdf. Berberine: Therapeutic potential of an alkaloid found in several medicinal plants (PDF). Alternative Medicine Reviews, **2(2)**, 94-103 **(1997)**.

[10] J.Peter, R.Gibbs Kenneth, Seddon; http://www.thorne.com/altmedrev/.fulltext/5/2/175.pdf. Berberine. Alternative Medicine Review London: British Library, ISBN 0712306498.PMID 10767672, **5(2)**, 175-7 **(2000)**.

[11] Y.Xu; Proteomic analysis reveals a synergistic mechanism of fluconazole and berberine against fluconazole-resistant Candida albicans: Endogenous ROS augmentation. Journal of Proteome Research, November, **8(11)**, 5296-304 **(2009)**.

[12] Poster Presentations. FEBS Journal. **277**, 37 **(2010)**.

[13] Y.Li, G.Y.Zuo; Advances in studies on antimicrobial activities of alkaloids. Chinese Traditional and Herbal Drugs, **41(6)**, 1006-1014 **(2010)**.

[14] F.R.Stermitz, P.Lorenz, J.N.Tawara, L.A.Zenewicz, K.Lewis; http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=26451. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc.Natl.Acad. Sci.USA., February, **97(4)**, 1433-7 **(2000)**.

[15] Zhang Shulin, Zhang Bo, Xing Kezhi, Zhang Xiumei, Tian Xiuping, Dai Wei; Inhibitory effects of golden thread (Coptis chinensis) and berberine on microcystis aeruginosa. Water Science & Technology, **61(3)**, 763 **(2010)**.

[16] O.P.Babbar, V.K.Chhatwal, I.B.Ray, M.K.Mehra; Effect of berberine chloride eye drops on clinically positive trachoma patients. The Indian Journal of Medical Research, PMID 7185757, **76**, 83-8, **(1982)**.

[17] G.Kalla, M.K.Singhi, Kalla Gyaneshwar; http://www.doaj.org/doaj?func=abstract&id=229883. Cutaneous leishmaniasis in Jodhpur district. Indian Journal of Dermatology, Venereology and Leprology, **62(3)**, 149-51 **(1996)**.

[18] J.Y.Zhou, S.W.Zhou, K.B.Zhang; Chronic effects of berberine on blood, liver glucolipid metabolism and liver PPARs expression in diabetic hyperlipidemic rats. Biological & Pharmaceutical Bulletin, **31(6)**, 1169-76 **(2008)**.

[19] http://en.wikipedia.org/wiki/Berberine

[20] http://en.wikipedia.org/wiki/Phosphoric_acid

[21] J.Srajer, R.Pindak, J.S.Patel; Phys.Rev.A, **43**, 126 **(1991)**.

[22] Y.Takanishi, Y.Ouchi, H.Takezoe, Jpn.Fukuda; J.Appl.Phys., **28**, L487 **(1989)**.

[23] Y.Ouchi, Y.Takanishi, H.Takezoe, Jpn.Fukuda; J.Appl.Phys., **28**, 2547 **(1989)**.

[24] Nagappa, S.K.Nataraju, D.Krishnamurthy; Mol.Cryst.Liq.Cryst., **31**, 133 **(1986)**.

[25] A.J.Slaney, J.W.Goodby; Liquid Crystals., **9**, 849 **(1991)**.

[26] M.Marthandappa, Nagappa, R.Somashekar, K.M.Lokanatha Rai; Phys.Stat.Sol.(a), **129**, 389 **(1992)**.

[27] M.Marthandappa, Nagappa, K.M.Lokhanatha Rai; J.Phys.Chem., **95**, 6369 **(1991)**.

[28] P.G.de Gennes; Solid State Commun, **10**, 753 **(1972)**.

[29] P.G.de Gennes, J.Prost; The Physics of Liquid Crystals,Clarendon Press, Oxford, **(1975)**.

[30] Nagappa, K.N.Jagadish, A.Shivaprasad, J.Mahadeva, P.R.Alapati; Mol.Cyst.Liq.Cryst, **301**, 1 **(1997)**.

[31] H.E.Neugebauer, J.Canad; J.Phys, **32**, 1 **(1954)**.

[32] W.Z.Helfrich; Naturfersch, **33a**, 305 **(1978)**.

[33] P.G.de Gennes; J.Phys.Paris, Colloque, **4**, 64 **(1969)**.

[34] P.G.de Gennes, J.Prost; The Physica of Liquid Crystal, Clarendon Press, Oxford, UK, 103 **(1998)**.