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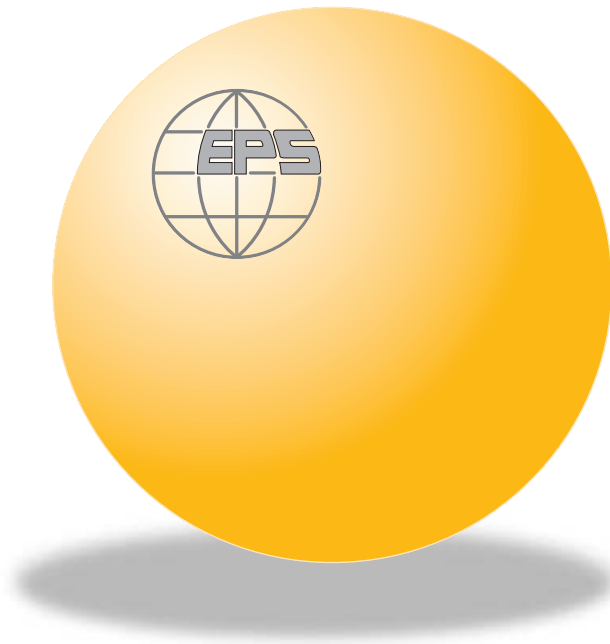
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with DNA and other polyelectrolytes**

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R. KRISHNASWAMY, P. MITRA, V. A. RAGHUNATHAN and A. K. SOOD



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## Tuning the structure of surfactant complexes with DNA and other polyelectrolytes

R. KRISHNASWAMY<sup>1</sup>, P. MITRA<sup>2</sup>, V. A. RAGHUNATHAN<sup>1</sup> and A. K. SOOD<sup>2</sup>

<sup>1</sup> *Raman Research Institute, Bangalore 560 080, India*

<sup>2</sup> *Department of Physics, Indian Institute of Science - Bangalore 560 012, India*

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**Abstract.** – We have carried out small-angle X-ray diffraction studies on complexes formed by the anionic polyelectrolytes, namely, sodium salts of double and single stranded (ds and ss) DNA, poly(glutamic acid) (PGA), poly(acrylic acid) (PAA), and poly(styrene sulfonate) (PSS) with a cationic surfactant system consisting of cetyltrimethylammonium bromide (CTAB) and sodium 3-hydroxy-2-naphthoate (SHN). All complexes have a two-dimensional (2D) hexagonal structure at low SHN concentrations. DNA-CTAB-SHN complexes exhibit a hexagonal to lamellar transition near the SHN concentration at which CTAB-SHN micelles show a cylinder to bilayer transformation. On the other hand, PGA and PAA complexes form a 2D centered rectangular phase at higher SHN concentrations, and PSS complexes show a primitive rectangular structure. These results provide a striking example of polyion specificity in polyelectrolyte-surfactant interactions.

Polyelectrolytes in aqueous solutions form complexes with oppositely charged surfactants, which have intriguing mesophase structures of great interest for industrial and biomedical applications [1]. The entropy gained by the release of counter-ions from the polymers and surfactants drives this complexation [2, 3]. X-ray diffraction studies of ds DNA with double-tailed cationic lipids reveal lamellar or 2D hexagonal structures depending on the lipids used, with the DNA strands intercalated between the bilayers in the lamellar phase [4, 5]. In the fluid  $L_\alpha$  phase the DNA strands are macroscopically oriented, while in the lower-temperature gel ( $L_{\beta'}$ ) phase they are translationally ordered as well, into a centred rectangular lattice [6]. Increasing bilayer flexibility or inducing a negative spontaneous curvature of the lipid-water interface results in hexagonally packed, lipid-coated DNA strands [5].

In this letter we present the results of small-angle X-ray diffraction studies on mixed surfactant-polyelectrolyte complexes. The surfactant system used was a mixture of the single-chained surfactant CTAB, which forms cylindrical micelles in dilute solutions [7], and SHN. The hydrotope SHN was added to CTAB in order to tune the shape of the micellar aggregates, as it is known to decrease the spontaneous curvature of CTAB micelles, resulting in a cylinder-to-bilayer transformation at  $[\text{SHN}]/[\text{CTAB}] \sim 0.64$  [8]. In the case of the DNA complexes, we find a hexagonal-to-lamellar transition at a SHN concentration of  $\sim 0.6$ , which suggests that the morphology of surfactant aggregates remains unchanged in the complex. Hence we conclude that the hexagonal complex has the intercalated structure shown in fig. 1A. The lamellar structure formed at higher SHN concentrations is similar to that found in the case of

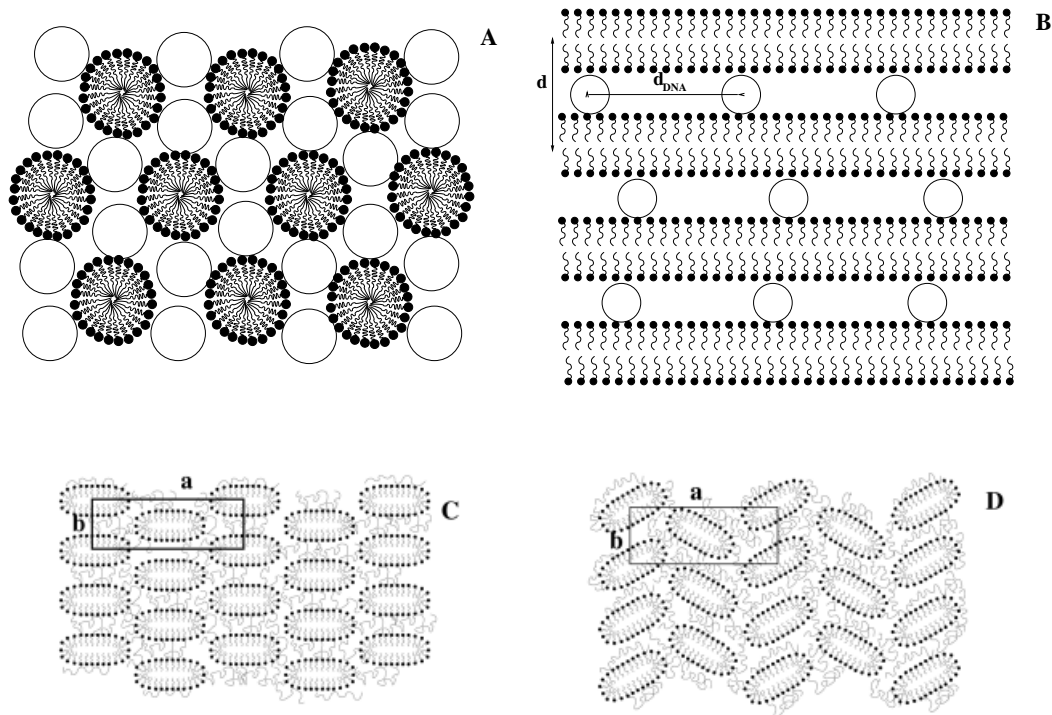


Fig. 1 – Schematic diagrams of the different structures observed in the present study. (A) The intercalated hexagonal phase, where each DNA strand is surrounded by three cylindrical micelles. The lattice parameter,  $a = \sqrt{3}(R_m + R_{DNA})$ , where  $R_m$  is the radius of the cylindrical micelle ( $\sim 2.0$  nm) and  $R_{DNA}$  that of a hydrated DNA strand ( $\sim 1.25$  nm). (B) The lamellar phase of DNA-surfactant complexes. (C) The centred rectangular  $cmm$  phase seen in PGA and PAA complexes at higher SHN concentrations. (D) The rectangular  $pgg$  phase observed in PSS complexes at intermediate SHN concentrations. In the structures shown in (C) and (D), ribbon-like surfactant aggregates are bridged by polymer chains.

double-chained lipids (fig. 1B). The complexes of PGA, PAA, and PSS also exhibit a hexagonal phase at low SHN concentrations. However, surprisingly, PGA and PAA complexes form a centered rectangular  $cmm$  structure and not a lamellar phase at higher SHN concentrations (fig. 1C), and PSS complexes exhibit a rectangular  $pgg$  structure (fig. 1D). These results indicate that the chemical nature of the polyion is an important parameter in determining the structure of the complexes.

Our interest in polyelectrolyte complexes with single-chained surfactants arises from earlier observations of a variety of structures in these systems. Depending on the surfactant concentration and the flexibility of the polyelectrolyte, these complexes are found to form cubic or hexagonal structures [9–11]. The length of the surfactant chain and the polyelectrolyte charge density are also known to influence the structure of these complexes [11]. Lamellar and cubic phases have also been observed in these systems when dried [12]. Although most of the studies have been on complexes of anionic polyelectrolytes with cationic surfactants, there have also been some studies on the complexes of cationic polyelectrolytes and anionic surfactants [13]. The single-chained cationic surfactant CTAB has been widely used for RNA and DNA extraction from plant cells and lambda phages [14]. Though complexation of CTAB with DNA is

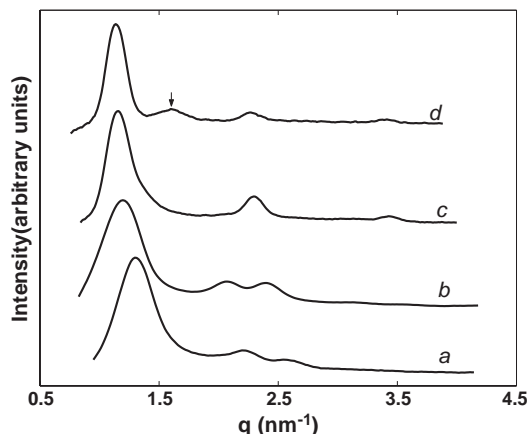


Fig. 2 – Diffraction patterns of the DNA-surfactant complexes.  $\alpha$  ( $=[\text{SHN}]/[\text{CTAB}]$ ) and  $\rho$  ( $=$  wt. of CTAB/wt. of polyelectrolyte) for the different curves are: 0, 1.0 (a); 0.55, 14.4 (b); 0.7, 14.4 (c); 0.7, 3 (d). The arrow on curve d indicates in-plane DNA-DNA correlation peak.  $\rho_{\text{iso}} = 3.74$  at  $\alpha = 0.7$ . CTAB concentration in the aqueous solution was 10 mM.

well known, the structure of these complexes has not been unambiguously established. X-ray diffraction studies indicate that they form a 2D hexagonal lattice, but the packing of the DNA strands and surfactant micelles within such a lattice has not been unambiguously determined, though a model has been proposed where DNA strands are intercalated between cylindrical CTAB micelles [15].

Sodium salts of Calf thymus ds DNA (30–50 kbp) and PGA (MW = 13650) were purchased from Sigma. CTAB, PAA (MW = 2000), 3-hydroxy-2-naphthoic acid (HNA), and sodium salt of PSS (MW = 70000) were obtained from Aldrich. M13 mp 18 ss DNA (7250 bp) was obtained from Bangalore Genei. Sodium salts of PAA and HNA were prepared by adding equivalent amounts of NaOH to the acid solutions in water and ethanol, respectively. Other chemicals were used as received. CTAB-SHN solutions with appropriate CTAB and SHN concentrations were first prepared in deionized water (Millipore). The relative SHN concentration,  $\alpha = [\text{SHN}]/[\text{CTAB}]$ , was varied from 0 to 0.7. The polyelectrolyte was then added to the surfactant solutions and the complexes, which precipitate out, were transferred into a 1 mm diameter glass capillary along with some of the supernatant for X-ray diffraction studies. The relative concentration of the polyelectrolyte,  $\rho = (\text{weight of CTAB})/(\text{weight of polyelectrolyte})$ , was varied over a wide range about the isoelectric point,  $\rho_{\text{iso}}$ , where the positive charges of the  $\text{CTA}^+$  ions are balanced by the  $\text{HN}^-$  ions and the negative charges on the polyelectrolyte.  $\text{Cu } K_{\alpha}$  radiation from a rotating anode X-ray generator (Rigaku, UltraX 18) operating at 50 kV and 80 mA was used to produce the diffraction patterns, which were collected on an image plate (Marresearch). Typical exposures lasted 2 to 3 hours.

DNA-CTAB-SHN complexes are found to be birefringent under a polarizing microscope. The diffraction pattern of the SHN free system shows 3 peaks in the small-angle region (fig. 2a). The magnitudes of their scattering vectors,  $q$ , are in the ratio  $1:\sqrt{3}:2$ , corresponding to the (1, 0), (1, 1) and (2, 0) reflections from a 2D hexagonal lattice with lattice parameter  $a = 5.64 \pm 0.09$  nm. The peak positions and their relative intensities are independent of  $\rho$ , and CTAB concentration up to 300 mM. There are two possible ways of packing DNA strands and CTAB micelles in a 2D hexagonal lattice, one of which is the intercalated phase shown in fig. 1A, and the other is the inverted hexagonal phase seen in some DNA-lipid complexes [5].

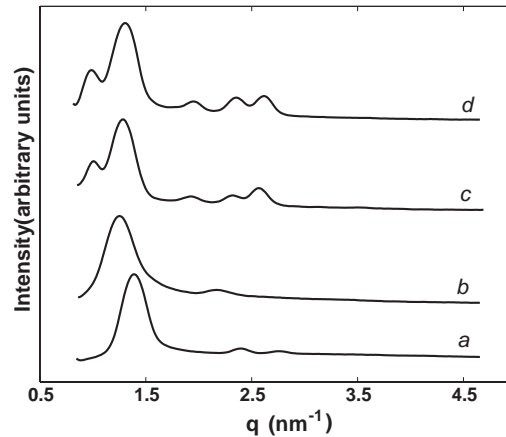


Fig. 3 – Diffraction patterns of the PAA-surfactant complexes.  $\alpha$  and  $\rho$  for the different curves are: 0, 6.0 (a); 0.4, 0.6 (b); 0.6, 12.0 (c); 0.6, 0.84 (d).  $\rho_{\text{iso}} = 9.0$  at  $\alpha = 0.6$ . CTAB concentration in the aqueous solution was 10 mM.

Neither of them can be ruled out on the basis of the observed values of  $a$ . The hexagonal phase persists in the presence of SHN, with  $a$  increasing gradually to 6.06 nm at  $\alpha = 0.55$  (fig. 2b). For  $\alpha > 0.6$  a lamellar complex is obtained (figs. 2c and d). This SHN concentration is comparable to that at which the micelles go from cylinders to bilayers [8]. Hence in this system, as in DNA-lipid systems reported in the literature, the structure of the complex is determined primarily by the morphology of surfactant aggregates. We may conclude that these complexes have an intercalated hexagonal structure, where each DNA strand is surrounded by three cylindrical micelles (fig. 1A). Moreover, if these complexes had an inverted hexagonal structure, decreasing the spontaneous curvature of the micelles by adding SHN would have further stabilized it [16]. There would have been no reason for it then to transform into a lamellar structure, as electrostatics alone would prefer the inverted hexagonal structure over the lamellar one. This is due to maximal neutralization of the DNA charges by surfactant ions resulting from the proximity of surfactant ions and polyions in the former case.

Structure of the lamellar DNA complex is similar to that found in DNA-lipid systems, with a periodicity of  $\sim 5.5$  nm (fig. 1B) [4]. At DNA concentrations below the isoelectric point ( $\rho_{\text{iso}} = 2.81$  at  $\alpha = 0.6$ ), we could not observe a peak corresponding to the DNA-DNA separation in the bilayer plane, probably due to its proximity to the strong first-order lamellar peak (fig. 2c). However, at higher DNA concentrations it is clearly seen in the diffraction pattern (fig. 2d). Our observations are consistent with a rapid change in the DNA-DNA spacing near the isoelectric point, as found in DNA-lipid complexes [17].

PAA-CTAB-SHN complexes are also birefringent and the diffraction pattern of the SHN free complex corresponds to a 2D hexagonal lattice, with  $a = 5.17 \pm 0.08$  nm (fig. 3a). The hexagonal phase is stable up to  $\alpha \sim 0.5$ .  $a$  increases gradually with SHN concentration and is equal to  $5.64 \pm 0.08$  nm at  $\alpha = 0.4$  (fig. 3b). It is independent of both CTAB concentration and  $\rho$ . (However, at very low CTAB concentrations ( $\sim 1$  mM) an optically isotropic complex is obtained, in agreement with earlier reports [10].) A very different type of diffraction pattern is obtained for  $\alpha > 0.5$ , which cannot be indexed on either a 2D hexagonal lattice or a lamellar lattice (figs. 3c and d). For example, diffraction pattern obtained at  $\alpha = 0.7$  and  $\rho = 0.72$ , shows five reflections with the following spacings: 6.23, 4.86, 3.27, 2.70, and 2.43 nm. These can be indexed as the (02), (11), (31), (20), and (22) reflections from a centered rectangular

lattice corresponding to the plane group  $cm\bar{m}$ , with lattice parameters  $a = 12.66$  nm and  $b = 5.40$  nm (fig. 1C). All diffraction patterns obtained at  $\alpha > 0.5$  can be indexed on similar lattices, with the lattice parameters showing weak dependence on SHN concentration and no dependence on  $\rho$ . Such a phase has not been observed in any of the polyelectrolyte-surfactant systems studied until now.

Structure of PGA-CTAB-SHN complexes are very similar to that of PAA complexes. For  $\alpha < 0.5$ , they exhibit a 2D hexagonal phase with  $a$  increasing gradually with  $\alpha$ , being  $5.33$  and  $5.84 \pm 0.08$  nm at  $\alpha = 0$  and  $0.4$ , respectively. At higher SHN concentrations these complexes show a centered rectangular phase. The lattice parameters  $a = 12.86$  nm and  $b = 5.48$  nm at  $\alpha = 0.6$  and  $\rho = 3.6$ . As in the case of PAA complexes, these parameters are insensitive to  $\rho$ , but depend weakly on SHN concentration.

PSS-CTAB-SHN complexes also form a hexagonal phase at low SHN concentrations. As in other cases,  $a$  increases with  $\alpha$ , from  $4.64$  nm at  $\alpha = 0$  to  $5.05 \pm 0.05$  nm at  $\alpha = 0.2$ . For  $\alpha$  between  $\sim 0.4$  and  $\sim 0.6$ , the peaks in the diffraction patterns can be indexed on a rectangular lattice corresponding to the plane group  $pgg$  (fig. 1D). The lattice parameters  $a$  and  $b$  vary from  $9.34$  and  $5.36$  nm at  $\alpha = 0.5$  to  $9.70$  and  $5.56$  nm at  $\alpha = 0.6$ . At higher SHN concentrations yet another phase is formed. The diffraction patterns of this phase contain only three peaks in the small-angle region, with no specific relationship between the corresponding values of  $q$ , and hence we have not been able to determine its structure unambiguously.

The hexagonal phase of PGA, PAA and PSS complexes with CTAB-SHN can be expected to consist of cylindrical micelles bridged by polyelectrolyte chains, as in the case of PAA-CTAB complexes [10]. The increase in the lattice parameter with SHN concentration is almost certainly a consequence of an increase in the micellar radius due to a reduction in the spontaneous curvature of the micelles. The structure of the centered rectangular phase seen at higher SHN concentrations in these systems is different from that of a similar phase seen in some DNA-lipid complexes [6]. In the latter case, such a lattice arises from transbilayer positional correlations between DNA strands. Consequently, the lattice parameter corresponding to DNA-DNA separation in the plane of the bilayer changes significantly as DNA concentration is varied across the isoelectric point. On the other hand, lattice parameters of the rectangular phase of PGA and PAA complexes are very similar and do not depend on the polyelectrolyte concentration. In fact correlations between PGA strands have not been observed in PGA-lipid lamellar complexes, even with contrast variation neutron scattering techniques [18]. Therefore, it is clear that PGA and PAA complexes under study have a structure very different from that of DNA-lipid complexes. A structure that seems reasonable is one consisting of ribbon-like aggregates arranged on a 2D rectangular lattice (fig. 1C), as found in some surfactant systems in between the hexagonal and lamellar phases [19]. It is very likely that the  $pgg$  phase seen in PSS complexes at high SHN concentrations also consists of similar aggregates (fig. 1D). Such structures have also been seen in surfactant systems [20].

The persistence length of a polyelectrolyte is very sensitive to counter-ion and salt concentrations [21]. Since most of the polyelectrolyte charges are neutralized in the complexes, the relevant quantity here is the intrinsic persistence length due to the stiffness of the polymer backbone. The persistence lengths of ds DNA, PGA, PAA, and PSS, obtained from the literature, are about  $50$ ,  $2$ ,  $1$ , and  $10$  nm. They carry a bare charge of  $1e^-/0.17$  nm,  $1e^-/0.154$  nm,  $1e^-/0.32$  nm, and  $1e^-/0.25$  nm, respectively. The above-quoted values may not strictly correspond to the present experimental conditions; nevertheless, it is clear that ds DNA differs widely from the other polyelectrolytes in its flexibility, whereas their bare charge densities are comparable. In order to see if the observed differences in the structure of the complexes at high SHN concentration is related to differences in their flexibilities, we have studied complexes formed by ss DNA with the same surfactant system. ss DNA has a persistence length of about

1.5 nm and a bare charge of  $1e^-/0.59$  nm. Interestingly, the structure of these complexes is similar to that of complexes of ds DNA. In particular, a lamellar structure with a periodicity of  $\sim 5.1$  nm is observed at high SHN concentrations. Hence it is clear that the formation of the non-lamellar phases at high SHN concentration in PGA, PAA, and PSS complexes is not related to either the flexibility or the charge density of these polymers.

The charged moieties in ds and ss DNA are phosphate groups, and those in PGA and PAA are carboxylate groups. PSS contains charged sulfonate groups. The fact that complexes of ds and ss DNA exhibit a lamellar structure at high SHN concentrations, whereas those of PGA and PAA show a *cmm* structure and PSS complexes a *pgg* structure, strongly suggests that the chemical nature of the polyion is responsible for the differences between these systems. These results pose a challenge to theories of polyelectrolyte-surfactant complexation, which presently cannot account for the variety of structures reported here.

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