

Recognition of Thymine Attached Cryptand with Adenine Functionalized Amphiphiles: Syntheses and Monolayer Study at the Air-Water Interface

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Abstract

Three ligands containing thymine attached through propyl spacer to the N atom(s) of a laterally non-symmetric cryptand (L_1 - L_3) and three 9-alkyladenine (A_1 - A_3 , alkyl chain lengths: C_{18} , C_{20} , C_{22}) amphiphiles were synthesized and characterized. Monolayers of the amphiphiles and amphiphiles mixed with thymine-cryptands were studied at the air-water interface on a Langmuir Blodgett Trough; molecular recognition between the amphiphiles and thymine-cryptands at the air-water interface was demonstrated from the change in the patterns of the pressure-area isotherms. Multi-layer Langmuir-Blodgett (LB) films were deposited on quartz plates and studied microscopically.

Keywords

Cryptand, Amphiphile, Molecular Recognition, Monolayers, Langmuir-Blodgett Film, Adenine, Thymine, Thin Film

1. Introduction

Synthetic amphiphiles are known to form stable molecular monolayers at the air-water interface on a Langmuir-Blodgett trough those can be transferred through LB technique [1] to construct monolayer or multilayered thin films of molecular dimension. Thus, formed films could be of highly ordered to be considered as twodimensional crystals of the amphiphiles and are important as materials with tailored interfacial properties [2]. In general, simplest amphiphiles containing alkyl chains composed of 12 or more carbon atoms could form monolayer on a LB Trough however, they should be nonvolatile, and insoluble in the sub-phase. In a real experimental setup, a minute amount of the amphiphiles are spread slowly on the surface of the sub-phase (water) in an LB trough so that the hydrophilic headgroups of the amphiphiles are attracted towards the sub-phase, and their hydrophobic tails directed towards the air on the LB trough. Initially, the amphiphiles remain in a relaxed arrangement (two-dimensional gas analogous phase, Figure 1A) while float freely lying flat on the surface. Compression of the surface results in a decrease in the surface area forcing the amphiphiles to pack closely (liquid-analogous phase, Figure 1B), and consequently the surface pressure (Π) of water increases. More compression results in the monolayer entering the region of solid/liquid coexistence (Figure 1C) where some of the alkyl chains of the lipids form twodimensional crystals (solid-analogous) but remain in a liquid-analogous lipid matrix. Continuous compression results in a highly-organized molecular monolayer (solid analogous or 2D crystal of the amphiphiles, Figure 1D) with decreased compressibility which is obvious from the steep rise in the surface pressure. Further compression leads

to an inflection point in the pressure-area isotherm when the monolayer collapses into bilayer or multilayer. The important indicator of the monolayer properties of a material is shown by a plot of surface pressure *versus* area of surface available to each molecule, known as pressure-area (Π -A) isotherm, when the compression of the surface, is carried out at a constant temperature. Organized molecular monolayers provide unique environments for molecular interactions and consequently for molecular recognition studies at the air-water interface.

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Figure 1. Cartoon diagram of (A) gas analogous, (B) liquid analogous, (C) solid–analogous in a liquid–analogous lipid matrix, and (D) solid–analogous phases of a monolayer

The study of the mutual recognition [3-5] of the complementary bases of nucleic acid (viz. adenine, thymine) in model systems, known to proceed spontaneously with high selectivity, has gained high attention because the nature selected their sequences as the most efficient mechanisms of accumulating, storing, reproducing and evolving genetic information [6], the basis of life. Information obtained by such studies may give much insight of the interactions which could be beneficial for genetic engineering research in addition to other interesting properties of such paired material recently came into light viz. bio-organic light emitting diode [7] as well. On the other hand, cryptands with various donor atom topologies could be the potential absorbents/ transporters for a specific guest (ionic and/or neutral small molecule), if properly designed to recognize the guest inside or outside of its cavity [8]. Again, nucleic base functionalized cryptand could be recognized when mixed with the complementary base-functionalized hydrocarbon chain leading to the formation of a supramolecular amphiphilic system. Study of such a supramolecular system could be important for many contemporary research areas either in chemistry or in biochemistry, viz. in understanding molecular interactions on biological cell surfaces [9], in applications such as OLED material, non-linear optical materials and chemical sensors [7, 10-12], in nanomaterial for biomedical application [13] and in achieving novel twodimensional molecular assemblies [14] composed of multiple chemical species. In simple words, the aim of the research was to combine two different system, namely an adenine-thymine system (able to store code) and a cryptand system (metal ion absorbent) so that the stability of the hydrogen-bonded system could be measured at the preliminary stage to develop smart devices.

Here, we present the syntheses of a series of thymineattached cryptand and 9-alkyladenine amphiphiles with different alkyl chain lengths, and studies of the monolayer of those supramolecular amphiphilic systems at the air-water interface on an LB Trough.



Figure 2. A synthetic strategy for L₃.



Figure 3. Schematic representation of synthetic route to the compounds L_1 , and L_2 .



Figure 4. Schematic representation of syntheses of $A_1 - A_3$.

2. Experimental Details

Materials and measurements

The cryptand L_0 [15, 16] and 1-(3-Bromopropyl)thymine [17] were synthesized following the reported methods. Thymine, di-*tert*-butyldicarbonate and 1,3-dibromopropane were obtained from Fluka (Germany). Hexamethyldisilazane and trimethylchlorosilane were obtained from Lancaster (UK). Reagent-grade anhydrous sodium sulfate, sodium carbonate, trifluoroacetic acid were received from Aldrich. The chemicals were used as received. All the solvents (Aldrich) were purified prior to the use following standard literature procedure [18]. De-ionized water used as sub-phase were obtained from Sigma.

¹H-NMR spectra were recorded on a JEOL JNM-LA400 FT (400 MHz) instrument in CDCl₃ with TMS as the internal

standard. FAB mass (positive ion) data were recorded on a JEOL SX 102/DA-6000 mass spectrometer. The electrospray mass spectra were recorded on an MICROMASS QUATTRO Quadruple Mass Spectrometer. Melting points were determined with an electrical melting point apparatus from PERFIT. The elemental analyses were carried out on a Perkin-Elmer 2400 CHN analyzer.

Monolayer and LB-Film

For monolayer studies, the sub-phase used was either deionized water or 1 M HCl. The II-A isotherms were obtained with computer-controlled Nima LB Trough (Nima Technology LTD, England). The instrument was mounted inside a dustfree acrylic box. Typically, the surface of the aqueous subphase was cleaned by aspiration until compression showed no surface contamination. Subsequently, 100 µL of a solution of amphiphile (1 mg/ml) in chloroform was spread on the top of the aqueous subphase to at least 20 sites using a micro-syringe. The period of time between spreading and compression was varied initially (15-60 min) to determine the result to be independent of this time interval. In subsequent experiments, a period of 30 min was typically used. A conventional linear extrapolation of the high-pressure limb in the liquid condensed region of the isotherm to zero pressure yields a limiting area per molecule, (A_0) . Reliability of the instrumental set-up was confirmed by examining monolayer compression and expansion curves of stearic acid (Sigma Chemical Co., USA). The minimum area per molecule obtained in our set-up was similar (25 Å²/ molecule) to the literature [19] value (25.5 Å²/molecule). Monolayer stability was investigated by monitoring the decrease in the area at constant surface pressure. Quartz plates were used as substrates for deposition of monolayers. The monolayers were transferred onto the substrates by a vertical dipping method. The surface morphology of the LB film deposited on quartz plates was probed with an Atomic Force Microscope (Molecular Imaging, USA). Surface profiler talystep (Alpha-Step 500 KLA Tencor) is used to measure the thickness of the thin films. A conical diamond tip of radius 0.0125 mm is used as a stylus. Force was adjusted to 2 mg. This method involves the movement of the stylus across a test groove or a step formed in the film. The step is made of the deposited film by removing a part of the film from the substrate using photoresist. The instrument measures the vertical movement of the stylus. When the stylus is encountered to the step, the vertical movement of it is amplified and the result is shown in the computer.

Syntheses

Mono- and bis- (BOC) protection on cryptand L_0 : L_M and L_B : Syntheses were carried out by following a method reported by this author earlier [20]. Di-tert-butyl dicarbonate (0.52 g, 2.4 mmol) was added to the solution of cryptand, L_0 (1.12 g, 2 mmol) in 50 ml of dichloromethane and stirred for 4 h at room temperature. The solvent was then evaporated to dryness. The mixed compounds were extracted with chloroform, washed with water; the organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated off to obtain a light brown mass. It was then subjected to column chromatography over silica gel (100-200 mesh) using chloroform and methanol as the eluent. The tris-BOC protected cryptand (L_T) comes out first with chloroform: methanol (99.5: 0.5% v/v) as the eluent. The *bis*and *mono*-BOC protected cryptands (L_B and L_M) can be eluted using (99: 1% v/v) and (98:2% v/v) chloroform: methanol respectively.

Mono-thymine cryptand, L_1 :

A suspension of 1-(3-bromopropyl)thymine (0.28 g, 1.1 mmol) in dry acetonitrile (50 ml) was stirred until a clear solution was obtained (~1 h). To this solution, L_B (0.76 g, 1 mmol) was added in the presence of excess triethylamine (0.25 g, 2.5 mmol) and stirred for 8 days. After this period, the solution became little turbid. After complete removal of the solvent, the yellow liquid left was shaken with 100 ml of water and the desired compound was extracted with chloroform (3 x 20 ml). The organic layer after drying over anhydrous sodium sulfate was evaporated off to obtain the desired compound as a brown solid. The solid dissolved in THF, stirred with 5 ml. of trifluoroacetic acid at room temperature for 12 h to remove the BOC protection. It was then neutralized with a saturated aqueous solution of sodium carbonate and sodium chloride; finally extracted with chloroform (3x20 ml). The chloroform extract was washed well with water (5x50 ml) and the chloroform was evaporated off completely to get a yellow solid after drying it over sodium sulfate. Pure L1 was obtained by recrystallization from ethanol as a light-yellow solid. Yield: 0.43 g (59%). Mp. 170°C. Anal. Calcd. for C₄₁H₅₅N₇O₅: C, 67.84; H, 7.64; N, 13.51%. Found C, 67.98; H, 7.69; N, 13.63%. ¹H-NMR (CDCl₃): δ 1.6 (s, 3H), 1.9 (m, 2H), 2.5 (m, 14H), 3.2 (br s, 4H), 3.5 (br s, 4H), 3.8 (br s, 2H), 4.2 (br s, 8H), 4.4 (br s, 2H), 7.2 (m, 13H). FAB-MS (m/z): 726 (70%) $[M]^+$.

Bis-thyime cryptand L₂:

1-(3-bromopropyl)thymine (0.55 g, 2.2 mmol) was allowed to react with L_M (0.62 g, 1 mmol) in presence of triethylamine (0.25 g, 2.5 mmol) in 50 ml dry acetonitrile and stirred for 8 days. The work up is same as described for synthesis of L_1 . Yield: 0.60 g (67%). Mp. 180 °C. Anal. Calcd. for C₄₉H₆₅N₉O₇: C, 65.97; H, 7.34; N, 14.13%. Found: C, 65.65; H, 7.19; N, 14.01%. ¹H-NMR (CDCl₃): δ 1.7 (m, 6H), 1.8 (br s, 6H), 2.45 (m, 12H), 3.2 (m, 4H), 3.4 (m, 2H), 3.6 (m, 8H), 3.8 (br s, 2H), 4.1 (m, 6H) 7.0 (m, 14H). FAB-MS (m/z): 892 (60%) [M]⁺.

Tris- thymine cryptand, L_3 :

To a solution of 1-(3-bromopropyl)thymine (1g, 4 mmol) in 50 ml of acetonitrile, the cryptand L_0 (0.56 g, 1 mmol) was added in presence of excess Et₃N and stirred for 8 days. Initially, a clear solution was obtained and after 6 days, a yellowish precipitate began to form. The precipitate was filtered off and washed with cold ethanol and crystallized from hot ethanol. Yield: 0.52 g (49%). Mp. 194°C. Anal Calcd. for C₅₇H₇₅N₁₁O₉: C, 64.69; H, 7.14; N, 14.56%. Found: C, 64.42; H, 7.01; N, 14.37%. ¹H-NMR (CDCl₃): δ 1.6 (m, 6H), 1.8 (br s, 9H), 2.5 (m, 12H), 3.2 (m, 6H), 3.5 (m, 6H), 3.7 (br s, 6H), 3.8 (m, 6H), 4.2 (m, 6H), 7.0 (m, 15H). FAB-MS (m/z): 1058 (100%) [M]⁺.

Adenine-amphiphiles $(A_1 - A_3)$:

All the amphiphiles were synthesized by reacting adenine with bromo-alkane in presence of sodium hydride base. In a typical synthesis of octadecyl derivative A_1 , a suspension of adenine (0.5 g, 3.7 mmol) in dry dimethyl formamide (50 ml) and sodium hydride (60% oil dispersion, 0.15g, 3.7 mmol) was treated with 1-bromooctadacane (1.23 g, 3.7 mmol). The mixture was stirred at RT for 24 h. The suspension was filtered off through Celite and evaporated under reduced pressure. The resulting slurry was diluted with water (100 ml) and extracted with CHCl₃ (3 x 50 ml). The extracts were mixed, dried over anhydrous sodium sulfate and the solvent was removed in a rotary evaporator. Crystallization from hot ethanol afforded the desired product as a white solid.

A₁. Yield: 1.05g, (74%). Mp. 73° C. Anal. Calcd. for $C_{23}H_{41}N_5$: C, 71.27; H, 10.66; N, 18.07%. Found: C, 71.09; H, 10.48; N, 18.21%. ¹H-NMR (CDCl₃): δ 8.34 (s, 1H), 7.77 (s, 1H), 5.82 (br s, 2H), 4.16 (m, 2H), 1.86 (br s, 2H), 1.22 (br s, 30H), 0.84 (t, 3H, J = 6.77 Hz)). FAB-MS: m/z 388 (100%) [M]⁺.

A₂. Yield: 1.1g, (72%). Mp. 88°C. Anal. Calcd. for C₂₅H₄₅N₅: C, 72.24; H, 10.91; N, 16.85%. Found: C, 72.01; H, 10.87; N, 16.71%. ¹H-NMR (CDCl₃): δ 8.32 (s, 1H), 7.72(s, 1H) 5.79 (br s, 2H), 4.1(m, 2H) 1.81(br s, 2H) 1.18 (br s, 34H), 0.8 (t, 3H, J = 6.71 Hz). FAB-MS: m/z 415 (100%) [M]⁺.

A₃. Yield: 1.15g, (70%). Mp. 106°C. Anal. Calcd. for $C_{27}H_{49}N_5$: C, 73.09; H, 11.13; N, 15.78%. Found: C, 72.89; H, 11.05; N, 15.72%. ¹H-NMR (CDCl₃): δ 8.30 (s, 1H), 7.73 (s, 1H), 5.73 (s, 2H), 4.11(m, 2H), 1.82 (t, 2H, *J* = 7.07 Hz), 1.18 (br s, 38 H), 0.81(t, 3H, *J* = 6.81 Hz). FAB-MS: m/z 444 (100%) [M]⁺.

3. Results and Discussion

Synthesis

Tris- thymine derivatized cryptand L_3 was obtained by allowing the 3-bromopropylthymine to react with the cryptand L_o in 3:1 ratio in dry acetonitrile in the presence of triethylamine as a base (Figure 2). However, L_1 and L_2 , mono- and bis- thymine derivatized cryptand were achieved in the stepwise syntheses, initially via the formation of BOCprotected cryptands, L_B and L_M; and further reaction of them with 3-bromopropylthymine in presence of triethylamine followed by the removal of protecting (BOC) unit. (Figure 3). The compounds could be purified by recrystallization from ethanol. On the other hand, the adenine-based amphiphiles, A1-A3, were synthesized by allowing alkyl bromides (C-18, C-20, C-22) to react with adenine in dry DMF in presence of sodium hydride as a base (Figure 4). The pure compounds were obtained by the recrystallization from hot ethanol. The ¹H-NMR spectra of the amphiphiles and the cryptand systems are consistent with their structures and the FAB-mass spectra exhibit the molecular ion peak in all cases signifying that the compounds are quite stable.

Monolayer Study

The shape of the pressure-area (Π -A) isotherm is subject to significant changes depending on the molecular structure of the amphiphiles, dissolved material, pH of the water subphase

[21]. However, when the pH remains constant, the shape of the curve is mainly influenced by the molecular structure.

It is well known that formation of the thymine-adenine conjugate takes place via attractive forces of intermolecular hydrogen bonding which again could have four possible binding modes [17, 19a, 22, 23] (Figure 5). Presumably, the strong hydrogen bonds between adenine and thymine lead to the formation of a giant supramolecular amphiphile upon mixing of one of the thymine attached cryptand (L_1 - L_3) with any one of amphiphiles (A_1 - A_3) where the cryptand unit behaves like a huge polar head-group and the hydrocarbon chain acts as a hydrophobic tail. The Π -A isotherms of the supramolecularly bound species would vary from the isotherms of individual components.



Figure 5. Possible binding modes of adenine and thymine derivatives.

For each amphiphile in the series A_1 - A_3 , a well-defined isotherm is found with the maximum pressure sustained by in excess of 60 mN/m (Figure 6). The high surface pressure sustained by each of these amphiphiles is attributable to the formation of highly ordered and well-packed monolayers. Moreover, the speed of compression either in the continuous mode or in quasi-static mode shows small hysteresis usually shown by well-behaved amphiphiles. The isotherms of the amphiphiles formed through association of a thymine derivative of cryptand (L₃) with 3 equivalent (with respect to the number of thymine groups attached to the cryptand) of one of the thymine-amphiphiles (A_1-A_3) demonstrate that in each case, the limiting area per molecule increases compared to pure thymine-amphiphiles (Figure 6a). Interestingly, the pattern of the isotherm of a pure adenine-amphiphile exhibits marked differences compared to that of the same amphiphile in presence of L₃. On the other hand, the maximum surface pressure that could be sustained by A₃ (>70 mN/m) is higher than that of A₂ (>50 mN/m) which is again able to sustain higher pressure than A_1 (<40 mN/m). This suggests that the number of carbon atoms of the amphiphile tail plays an important role in the packing on the air-water interface and the amphiphilic chain lengths could be organized according to their maximum sustainable pressure as $C_{22}>C_{20}>C_{18}$. Similar observation was noted for simple cryptand based systems reported earlier [20, 21].





Figure 6. Comparison of isotherms formed by (a) pure adenine-amphiphiles (A_1-A_3) and L_3 mixed with them in 3:1 molar ratio; (b) pure amphiphiles (A_1-A_3) and L_2 mixed with them in 2:1 molar ratio; (c) pure amphiphiles (A_1-A_3) and L_1 mixed with them in 1:1 molar ratio.

For non-interacting mixed amphiphiles limiting area/molecule is theoretically $a_{12} = n_1 a_1 + n_2 a_2$; $(n_1 \text{ and } n_2 =$ mole fractions of amphiphiles 1 and 2 in the mixture respectively; a_1 and a_2 = limiting area/molecule observed for pure amphiphiles 1 and 2 respectively) [21]. Although the cryptand derivatives, L_1-L_3 are insoluble in the water subphase, however, can sustain much lower pressure (>15 mN/m) at the air-water interface. The summation of the limiting area per molecule for 3 pure adenine amphiphile (Say for A_1) molecules and a pure cryptand based molecule $(say L_3) = [3A_1+L_3 = 3x42+15 = 137]$ is lower compared to that of the mixed system (160 mN/m) actually observed [Table 1]. An increase in the limiting area/molecule of the mixed system compared to that of a calculated one, is indicative of attractive forces playing their role and leading to the formation of supramolecular amphiphile formed between an adenine amphiphile (A_1) and a cryptand-based system (L_3) which affect orientation and packing of the A_1 at air-water interface. A cartoon diagram shown in Figure 7 could be helpful why we should expect the area/molecule would increase in presence of an attractive force. As all attempts to crystallize the mixed amphiphile remained unsuccessful, nothing more can be commented upon the structure of the associated species. A similar observation could be obtained while studying L_3 with either A_2 or A_3 [Table 1]. The maximum pressure sustained by the mixed system (L_3+3A_2) and (L_3+3A_3) are much lower than that of pure A_2 and A_3 respectively. However, a comparable maximum pressure sustained by A_1 and (L_3+3A_1) , that chain length of the amphiphilic tail could not overcome the presence of large head group to be packed well on the water surface at higher pressure.



Figure 7. Schematic representation of two component species at air-water interface (A) probable spatial arrangement of non-interacting species in their solid analogous phase. (B) probable spatial arrangement of two species when conjugation of adenine and thymine forms a supramolecular amphiphile occupying larger space at the air-water interface.

When the mixed system was composed of L_2 , the molar ratio of L_2 to A_n was maintained as 1:2. In a similar observation, it was found that a summation of area/molecule for pure components is lower than that of the mixed system (Figure 6b, Table 1) indicative of unfavorable packing arrangement of the supramolecular system compared to the pure A_1 - A_3 . Maximum sustainable pressure showed a similar trend as of described for the L_3 -incorporated mixed system. However, when the mixed system was made with L_1 , maintaining the molar ratio of L_1 to A_n as 1:1, L_1 + A_n system showed the same trend as described for L_2 and L_3 (Figure 6c). Although, the summation of limiting area/molecule for pure components are lower than that of the mixed system, but the difference is minimum in the whole series. Because of the presence of just one unit of thymine in L_1 , only one molecule

of A_n would form an associated species which could be packed much closely at the air-water surface as the headgroup orientation is less strained. The values of area/molecule of the associated amphiphiles are collected in Table 1 and illustrated in Figure 8 as a bar diagram.

Table 1. Limiting area $(A^2)/molecule$ at the air-water interface of the amphiphiles $(A_1 - A_3)$ and amphiphiles mixed with the proportionate equivalent of adenine derivatized cryptand systems $(L_1 - L_3)$.

Adenine-based	Limiting area/molecule for	Limiting area/molecule of	Limiting area/molecule of the	Limiting area/ molecule of
amphiphiles	pure amphiphile (in Ų)	the system (3A: L ₃) (in Å ²)	system (2A: L ₂) (in Å ²)	the system (A: L ₁) (in Å ²)
A ₁	42	160	122	74
A_2	32	110	82	54
A ₃	25	92	70	39



Figure 8. Bar chart of the limiting area/molecule (\hat{A}^2) of the monolayers formed by (i) (A): adenine based amphiphiles (A_1-A_3) (ii) (A+L1): (A_1-A_3) mixed with (L_1) in 1:1 molar ratio (iii) (2A+L2): (A_1-A_3) mixed with (L_2) in 2:1 molar ratio (iv) (3A+L3): (A_1-A_3) mixed with (L_3) in 3:1 molar ratio.

The stability (decrease in monolayer area at a constant pressure) for all of the supramolecular amphiphiles are less compared to that of the pure adenine based amphiphiles. However, if one compare among the mixed system (one ligand say L_1 is constant), stability decreases along with decreasing chain length A_3 (C-22)> A_2 (C-20)> A_1 (C-18) which is expected as the size of head-group: tail length is favorable for C-22 amphiphile. On the other hand, a comparison of all the L_1 - L_3 with respect to an adenine amphiphile (say A₂) reveals the stability of the monolayer decreases as mono-thymine-cryptand (L_1) bis-thyminecryptand (L₂)>tris-thymine-cryptand (L_3) which is attributable to the fact that strain increases as mixed amphiphile formed as $(A_2+L_1) < (2A_2+L_2) < (3A_2+L_3)$ at the air-water interface (Figure 9).

In presence of an acid sub-phase, the isotherm shows an expansion *i.e.* it attains the liquid analogous state at the larger surface area, though the limiting area per molecules remains almost comparable (Figure 10). This suggests that protonation of the amphiphilic head-groups generate repulsion among them and it attains the liquid analogous state early. But in the solid analogous phase, the effect of these repulsive forces are being nullified by a kind of attractive force operating among them existence of which has been indicated by the hysteresis of the isotherms.



Figure 9. A representative plot of the stability of the monolayers at the airwater interface for the A_2 and A_2 mixed with thymine derivatized cryptand systems.

Figure 10. Comparative representation of Π -A isotherm of the monolayer formed by A_2 mixed with L_2 in 2:1 molar ratio in pure water subphase (—) and 1M HCl sub-phase.

Deposition of Langmuir-Blodgett films

The monolayers at the air-water interface were transferred to quartz plate to deposit multi-layered Langmuir-Blodgett film. The deposition took place at each downward and upward stroke. Dipping speed was maintained at 2 nm/min. We have deposited pure A_2 and A_2 mixed with L_1 , L_2 and L_3 in appropriate molar ratio. Surface profiler talystep measurement revealed the thickness of 20 layers of mixed (in the ratio 1:1) amphiphile $(A_2 + L_1)$ was 580Å, whereas 30 layers of $(A_2 + L_1)$ had a thickness 820Å suggesting an average thickness of each layer as 29 Å and 27.3 Å. This difference in thickness could be addressed as adenine and thymine can competitively make H-bond with water, the film formed could be less ordered in case of the number of lavers increased. The single layer thickness is slightly more than an energy minimized C-20 chain length (24 Å). 20 layers of mixed (in the ratio 3:1) amphiphiles $(3A_2 + L_3)$ afforded 450 Å suggesting that a less compact packing arrangement could be achieved in a monolayer at water surface which ultimately results in a film with more defects in packing.

The AFM study by the non-contact method showed that the films had been formed with consistency. For example, root mean square (RMS) value of the surface roughness for 20 layers of $A_2 + L_3$ (3:1 molar ratio) was estimated to be 18 nm and that for $A_2 + L_1$ (1:1 molar ratio) was 9.2 nm when the AFM study was carried out at 1000 nm resolution. A representative AFM images are shown in Figure 11.



Figure 11. AFM images of LB films formed by deposition of 20 layers of mixed amphiphile consist of A_2 and L_3 in the molar ratio 1:3 at resolution 700 nm.

4. Conclusion

The amphiphiles (C-18, C-20, C-22) with adenine headgroup form highly stable monolayers at the air-water interface. A thymine derivatized cryptand when present with an adenine-amphiphile on the water surface, adeninethymine interaction causes them to form non-covalently bound supramolecular amphiphilic species which can be confirmed by the increase in the limiting area/molecule ($Å^2$) of the species which is even higher than the summation of the limiting area/molecule of the individual components. The reason is that the cryptand unit with thymine remain on water and acts as a large headgroup for the associated species and the adenine unit of A1-A3 comes close to the thymine-unit on cryptand forming H-bonds so that the amphiphilic-tail carbon chains conformation at ground state changes from its direction of a right angle onto water surface as in pure A_1 - A_3 . The maximum sustainable pressure for the mixed system is lower than that of pure A₂ and A_3 , though A_1 showed almost similar pressure sustainability for the pure and mixed system. Stability of the mixed monolayer, involving tris-derivatized cryptand, was found to be the least whereas that involved monoderivatized-cryptand was most. Presumably, tris-derivatized cryptand forms triple-tailed associated species with highest conformational strain. Guest-inclusion complexes can be synthesized with designer cryptands to functionalize similarly as in the present study which may endow ultrathin films with fascinating chemical, optical, electrical, thermal, and electro-optical properties [24, 25] as the LB technique can be effectively utilized to develop films with cryptand-based amphiphiles for having materials with desired interfacial properties. This can serve as a model study in understanding substituent influence on adeninethymine in the biological system [26] and may find potential use in an application like chromatography [27].

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