TWO-DIMENSIONAL CONDENSATION OF NUCLEOBASES: A COMPARATIVE STUDY OF HALOGEN DERIVATIVES OF CYTOSINE

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Dedicated to the 50th anniversary of rewarding the Nobel Prize to Professor Jaroslav Heyrovský for the discovery of polarography.

The high ability of self-association of nucleic acid components leads to a two-dimensional (2D) condensation at electrode surfaces. The driving force of the process resides in the intermolecular interactions, such as dispersion forces, hydrogen bonding or electrostatic interactions. In this study, the condensation of 5-halogen cytosine derivatives (5-fluorocytosine, 5-bromocytosine and 5-iodocytosine) at the hanging mercury drop electrode was investigated to evaluate the influence of the different types of intermolecular interactions in the phenomenon. All of these derivatives form 2D capacitance pits, but in distinct conditions of pH, concentrations and temperature. Dispersion forces are identified as the main contributor to the 2D condensation of 5-iodocytosine, while for 5-fluorocytosine this is hydrogen bonding in hemiprotonated dimers. The third derivative, 5-bromocytosine, is an intermediate case between the two formers.

Keywords: Cytosine derivatives; Pyrimidines; 2D condensation; Capacitance pit; Hanging mercury drop electrode; 5-Fluorocytosine; 5-Bromocytosine; 5-Iodocytosine; Electrochemistry.

Nucleic acid components (bases, nucleosides and nucleotides) are adsorbed at electrified interfaces in a broad region of electric potential. Due to their high propensity to self-association, nucleic acid bases are able to form on various substrates two-dimensional (2D) condensed monolayers. This phenomenon, initially evidenced at dropping mercury[1,2] has since been observed with other materials such as hanging mercury drop electrodes, mercury film electrodes[3–5], or gold[6–9] and silver[10] single crystals. Although, a number of neutral organic molecules form 2D condensed layers[11,12], nu-

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cleic acid bases differ from other purine and pyrimidine derivatives, not occurring in nucleic acids (like isocytosine, isoguanine, xanthine), which do not undergo 2D condensation. It has been suggested that this interesting physical property of nucleic acid components has probably played a significant role for the origin of life on Earth[13–15].

Two-dimensional condensed monolayers exhibit supramolecular organisations, characterised by a long-range ordering underlain by intermolecular interactions. The formation of ordered adlayer is a complex phenomenon, because different type of interactions can be involved in the stabilisation of the monolayer, such as hydrogen bonding between “flat-lying” adsorbates[16–18], stacking between “vertically” oriented molecules[19–27], dispersion[28,29] or electrostatic interactions[30–32]. Besides, dipole orientation in the electric field[1,2,21,22,29,30] can lead to reorientation processes at the interface, resulting for a given compound in the existence of various 2D condensed layers depending on the potential[23,30,31,33].

We present herein a comparison of the interfacial behaviour of three halogen derivatives of cytosine (5-fluorocytosine, 5-bromocytosine and 5-iodocytosine) at the hanging mercury drop electrode (HMDE). The adsorption of the cytosine derivatives has been studied by differential capacitance measurements, which is very sensitive to the interfacial structure of the nucleic acid adlayers[11,12]. When compact films are formed, the differential capacitance of the electrode double layer is considerably depressed, giving rise in the capacitance–potential (C_d–E) curves to characteristic “pits” (observed for the first time by Lorenz in 1958 with solutions of nonanoic acid[34]).

The aim of this work is to evaluate effects of halogen derivatives of cytosine on 2D condensation, to contribute to better understanding of the role of the different intermolecular interactions in the 2D condensation of nucleic acid components.

EXPERIMENTAL

A three-electrode system was used for the measurements. Platinum wire (diameter 1 mm) served as counter electrode, the reference electrode was Ag|AgCl|3 mol dm^{-3} KCl. The potentials given in this work refer to this scale. Measurements were done on HMDE system (Metrohm VA stand 663, Switzerland) with drop area of 0.4 mm^2. All measurements were performed with an AUTOLAB electrochemical system PGSTAT 302 (EcoChemie, Netherlands) equipped with a frequency-response analyzer module (FRA2). The C_d–E curves were measured (if not stated otherwise) at a frequency of 9943 Hz with an AC voltage amplitude of 10 mV. The reason for employing such a high ac perturbation frequency is to elude the faradaic response coming from the cytosine derivatives reduction (use of 9943 Hz has a reason to avoid integer multiple or simple fractions of 50 Hz commercial frequency, to avoid electrical interferences). Concentrations of cytosine derivatives were checked by Libra S22 spectrophotometer. The temperature (278 K if no other mention is done) of the thermostated cell was controlled by a Huber CC2-K6 thermostat-cryostat. For all measurements, Britton–Robinson buffer with 0.5 mol dm^{-3} NaCl and tri-distilled water were used. The buffer was modified with 2 mol dm^{-3} NaOH to given pH. All chemicals were highest purity available (Sigma–Aldrich, USA).

RESULTS

5-Fluorocytosine

Most of the studies on the condensation of nucleic acid components have been carried out at neutral pH, at which the 2D condensation of 5-fluorocytosine (5-FC) has never been observed. We show for the first time that a characteristic capacitance pit is obtained at the HMDE in acidic medium. Figure 1a shows the influence of the pH on the differential capacity curves recorded at 278 K and 45 mmol dm^{-3} concentration of 5-FC. At pH 4.4, the lower capacitance measured in the presence of 5-FC as compared to the pure electrolyte clearly indicates the adsorption of the surfactant in the potential range from –1.0 to –0.2 V, but no capacitance pit is detected. This latter appears when the pH is decreased below 4.1. Upon decreasing further the pH, the pit bottom reaches a constant capacitance value (the pit bottom) $C_d \approx 7.0$ μF cm^{-2} which is almost independent of the electrode potential, as expected for a condensed layer. In parallel, the pit width first increases then progressively shrinks until vanishing at pH < 2.1.

The compact character of the film is further evidenced by the concentration and temperature dependences. Figure 1b shows the influence of the former at pH 2.7. The 2D condensation requires a minimal 5-FC concentration (higher than 15 mmol dm^{-3}), the pit bottom is independent of both the potential and concentration, and the pit width increases with the concentration. At 55 mmol dm^{-3}, which is very close to the saturation concentration, a second capacitance depression is noticeable at less negative potential, indicating the formation of a quite dense film at these potentials. The temperature dependence (not shown) followed the trend expected for a 2D condensed layer, showing a decrease of the pit width upon increasing the temperature. The critical temperature T_c (i.e. the highest possible condensation temperature), determined by measuring the pit width at different temperatures and extrapolating to zero width, was found to be 302 ± 2 K for all pH values at a 45 mmol dm^{-3} concentration.

5-Iodocytosine

The 2D condensation of 5-iodocytosine (5-IC) has been reported at pH 7 by Retter[26], who employed a gas lattice model to evaluate the thermodynamical parameters of the compact film. We have searched for 2D condensation of 5-IC in a wider pH range, going from pH 3 to 11 (the same conditions were kept for 5-bromocytosine too). As it is obvious from Fig. 2, 5-IC forms at 278 K condensed layers for all selected pH values. The best pronounced 2D capacitance pit with sharp pit edges was obtained with

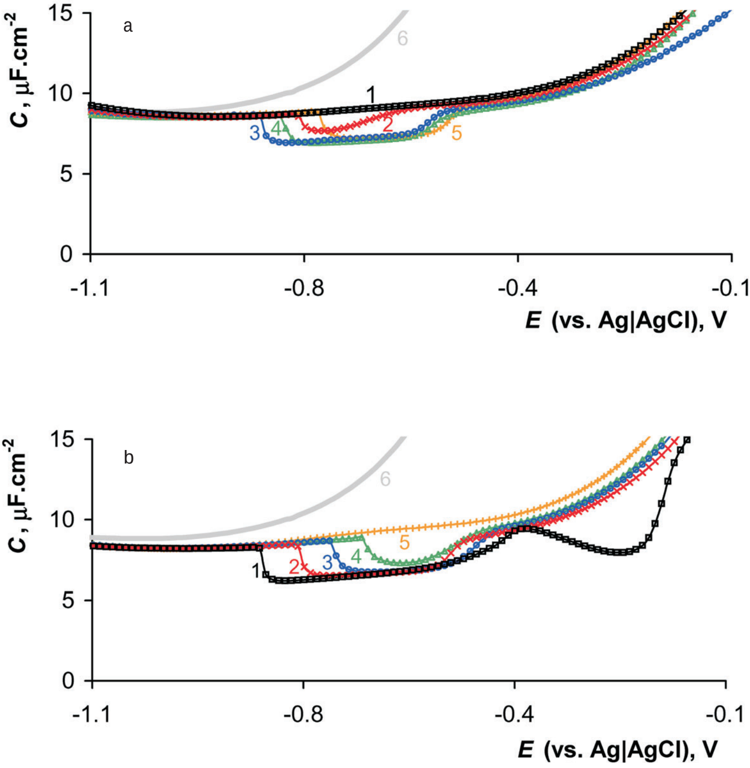

FIG. 1

a The plot of specific area capacitance, *C*, as a function of electrode potential, *E*, for HMDE at presence of 45 mmol dm^{-3} concentration of 5-fluorocytosine at 278 K in an electrolyte of various pH: 1 4.4 (black); 2 4.0 (red); 3 3.4 (blue); 4 2.7 (green); 5 2.2 (yellow); 6 blank electrolyte (grey solid line, pH 2.7). b The plot of specific area capacitance, *C*, as a function of electrode potential, *E*, for HMDE at presence of various concentrations of 5-flurocytosine (in mmol dm^{-3}) at 278 K in an electrolyte (pH 2.7): 1 55 (black); 2 40 (red); 3 30 (blue); 4 25 (green); 5 15 (yellow); 6 blank electrolyte (grey solid line)

3.5 mmol dm^{-3} in the conditions used by Retter[26], i.e. at pH 7. The minimal condensation concentration was found to be 0.4 mmol dm^{-3} and the critical temperature T_c = 320 ± 3 K, in very good agreement with the reported data[26].

Examination of Fig. 2 shows that 5-IC exhibits a diversity of behaviours at the HMDE, depending on the pH and applied potential. At pH 9 (Fig. 2c), a wide pit centred around –0.63 V is observed, having the typical characteristics associated with an ordered layer. While a complete desorption of 5-IC occurs at the negative end of the pit, capacity curves indicate the formation of another adlayer at potentials around –0.37 V. From the higher capaci-

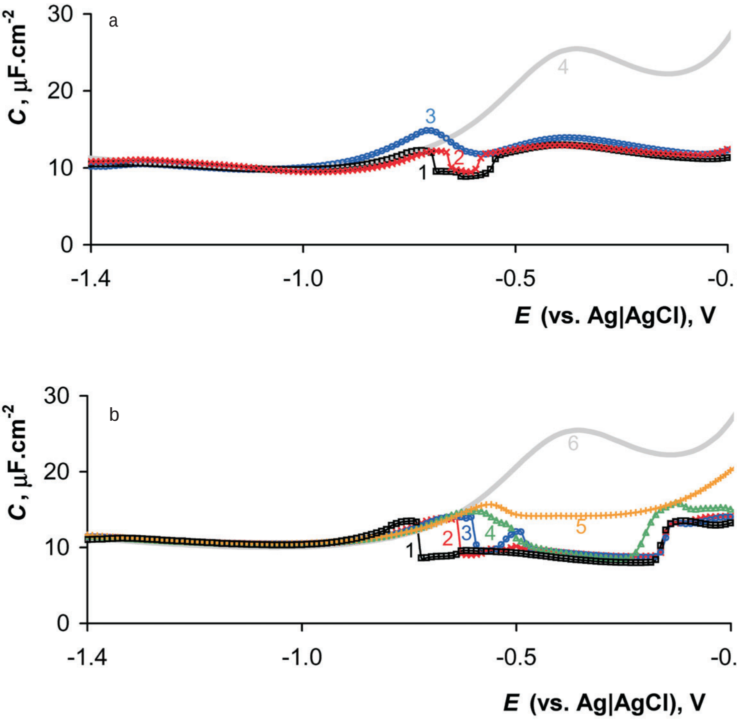

FIG. 2

The plot of specific area capacitance, *C*, as a function of electrode potential, *E*, for HMDE at presence of various concentrations of 5-iodocytosine (in mmol dm^{-3}) at 278 K in an electrolyte of different pH. a For pH 3.0: 1 4.5 (black); 2 3.0 (red); 3 1.8 (blue); 4 blank electrolyte (grey solid line). b For pH 5.0: 1 3.3 (black); 2 1.2 (red); 3 0.9 (blue); 4 0.6 (green); 5 0.3 (yellow); 6 blank electrolyte (grey solid line). c For pH 9.0: 1 3.0 (black); 2 1.5 (red); 3 1.0 (blue); 4 0.6 (green); 5 0.4 (yellow); 6 blank electrolyte (grey solid line). d For pH 11.0: 1 8.0 (black); 2 5.0 (red); 3 4.0 (blue); 4 2.0 (green); 5 0.3 (yellow); 6 blank electrolyte (grey solid line) tance value and the concentration dependence, it can be inferred that this film, which is desorbed at –0.49 V, is less dense than the former one, whose minimal condensation concentration and critical temperature were evaluated to be 0.4 mmol dm^{-3} and 315 ± 3 K, respectively.

In striking contrast to 5-FC, decreasing the pH tends to destabilise the adlayers of 5-IC. At pH 3 (Fig. 2a), only a very narrow, ill-defined pit is obtained, provided pretty high concentrations (> 2 mmol dm^{-3}) are used. This suggests that protonation of the nucleic base can have a destabilising effect in the case of 5-IC but a stabilising one in the case of 5-FC. This point will

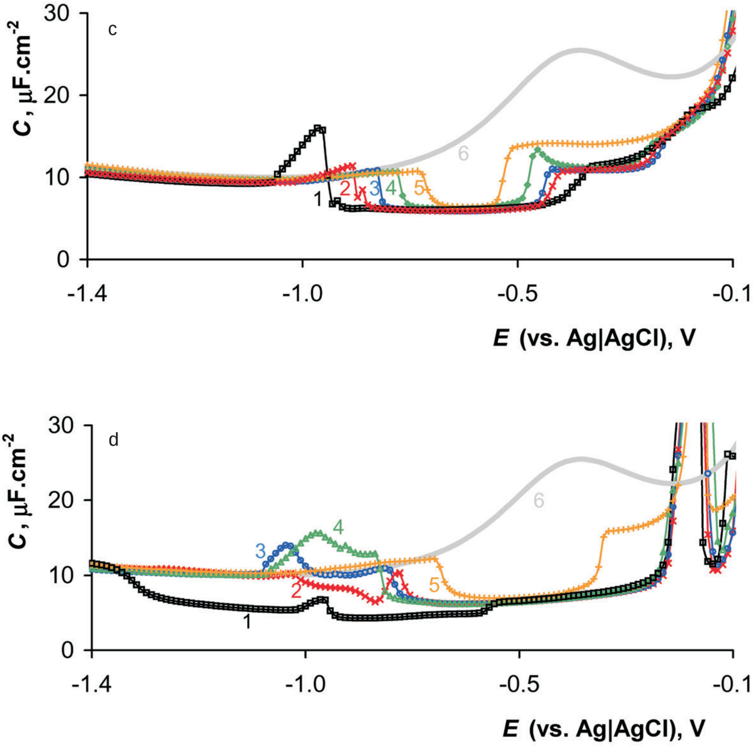

FIG. 2 (*Continued*)
The plot of specific area capacitance, *C*, as a function of electrode potential, *E*, for HMDE at presence of various concentrations of 5-iodocytosine (in mmol dm^{-3}) at 278 K in an electrolyte of different pH. a For pH 3.0: 1 4.5 (black); 2 3.0 (red); 3 1.8 (blue); 4 blank electrolyte (grey solid line). b For pH 5.0: 1 3.3 (black); 2 1.2 (red); 3 0.9 (blue); 4 0.6 (green); 5 0.3 (yellow); 6 blank electrolyte (grey solid line). c For pH 9.0: 1 3.0 (black); 2 1.5 (red); 3 1.0 (blue); 4 0.6 (green); 5 0.4 (yellow); 6 blank electrolyte (grey solid line). d For pH 11.0: 1 8.0 (black); 2 5.0 (red); 3 4.0 (blue); 4 2.0 (green); 5 0.3 (yellow); 6 blank electrolyte (grey solid line)

be discussed in the next section. At the intermediate pH 5 (Fig. 2b), two distinct films are formed, the first one appearing at the least negative potentials at 0.6 mmol dm^{-3} concentrations, while the second appears only at higher concentrations.

The deprotonation of 5-IC at pH 11 makes it much more soluble, and higher concentrations can be reached, at which complex adsorption processes could be observed (Fig. 2d). Up to 4 mmol dm^{-3}, only one well-defined pit is observed, then a second region of depressed capacitance starts to appear at the negative side of the pit, until three distinct films can be clearly distinguished at a concentration of 8 mmol dm^{-3}. A comparable situation was reported for high concentrations of 5-methylcytosine[32].

5-Bromocytosine

Like 5-IC, 5-bromocytosine (5-BC) has been reported to undergo 2D condensation at pH 7 (Retter[25]) and our results show that condensed layers are formed in the whole pH range between 3 and 11. The adsorption behaviour of 5-BC at pH 9 (Fig. 3b) is pretty similar to that of 5-IC, with a well-defined capacitance pit appearing at concentrations > 0.7 mmol dm^{-3}, a pit bottom at $C_d \approx 6.6$ μF cm^{-2} and a critical temperature $T_c = 305 \pm 3$ K (or 308 ± 3 K at pH 7). Here again, a second film occurs at less negative potentials, though only at concentrations close to the saturation. When the pH is increased beyond the pK_{a2} value (Fig. 3c), a pit is still discernible but its width is much smaller than in the previous case, probably due to electrostatic repulsions between deprotonated, negatively charged 5-BC molecules (the molecules are essentially protonated (thus positively charged) at $pH < pK_{a1}$, neutral at $pK_{a1} < pH < pK_{a2}$, and deprotonated at $pH > pK_{a2}$).

A very interesting behaviour is observed at pH 3 (Fig. 3a). A small pit located at –0.525 V appears at a 1.3 mmol dm^{-3} concentration of 5-BC, whose width increases in the negative potentials direction when the concentration increases. At even higher concentrations, around 4.6 mmol dm^{-3}, a second pit is formed, expanding in both potential directions. The growth of this second capacitance pit happens to the detriment of the first film, which completely disappears upon further increase of the concentration.

DISCUSSION AND CONCLUSIONS

The above results show that the nature of the substituent strongly affect the adsorption and two-dimensional condensation of cytosine derivatives at the HMDE, in terms of pH, potential, concentration and temperature. As al-

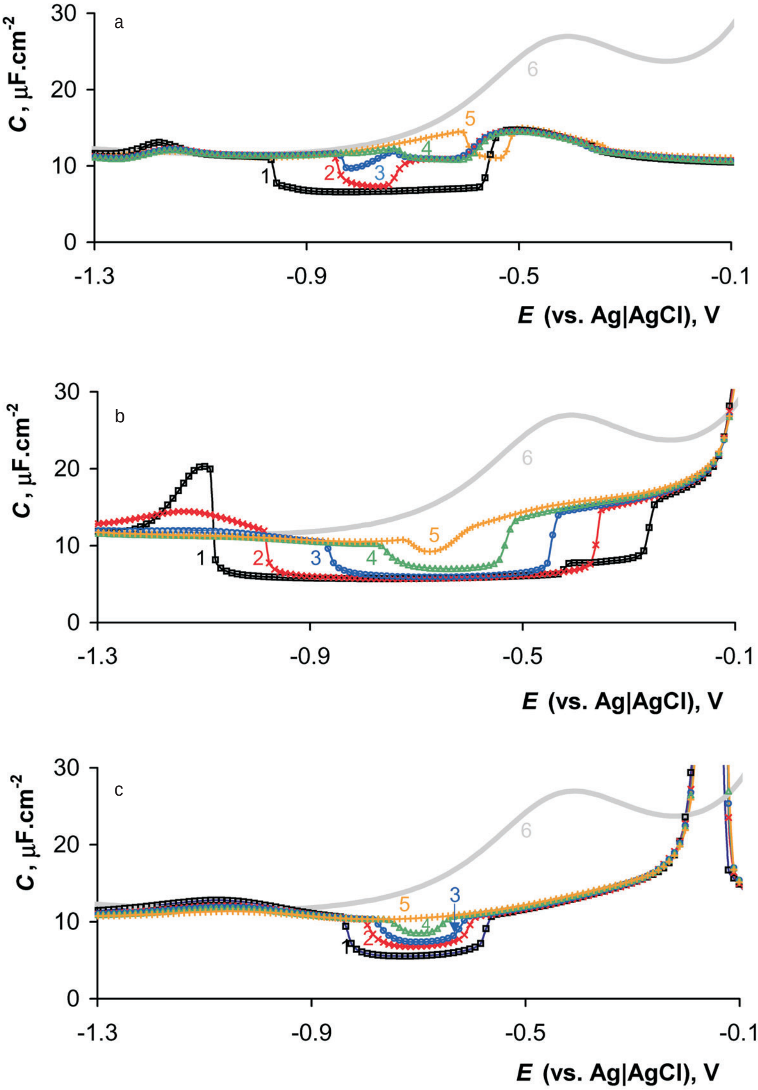

FIG. 3

The plot of specific area capacitance, *C*, as a function of electrode potential, *E*, for HMDE at presence of various concentrations of 5-bromcytosine (in mmol dm^{-3}) at 278 K in an electrolyte of different pH. a For pH 3.0: 1 10.0 (black); 2 5.0 (red); 3 4.7 (blue); 4 4.6 (green); 5 1.3 (yellow); 6 blank electrolyte (grey solid line). b For pH 9.0: 1 10.0 (black); 2 4.0 (red); 3 1.8 (blue); 4 1.0 (green); 5 0.8 (yellow); 6 blank electrolyte (grey solid line). c For pH 11.0: 1 10.0 (black); 2 8.0 (red); 3 6.0 (blue); 4 5.0 (green); 5 4.0 (yellow); 6 blank electrolyte (grey solid line) ready stated in the introduction, 2D condensation is intimately linked to the nature and amplitude of intermolecular interactions, and it is thus interesting to discuss our results in this light. Orientation of the permanent dipole moment in the electric field is unlikely to be a major energetic contribution in the present case. Indeed, all the condensed layers are obtained in potential ranges not far away (< 500 mV, a notable exception being 5-IC at pH 11) from the potential of zero charge, whereas dipole orientation-induced condensation of cytosine and other nucleic acid components occurs at potentials as negative as –1.3 V (Vetterl[21,22]). Various authors have focused on hydrogen bonding[18] and dispersion forces[28,29] to interpret 2D condensation phenomena and correlate theoretical estimations and experimental values of critical temperatures. Sangaranarayanan and coworkers[18,28] have assumed in both instances that one contribution was overwhelming the second one, a situation which is not valid in every experimental conditions.

The case of 5-FC is relatively simple, with only one well-defined pit (we do not treat here the peculiar case of 55 mmol dm^{-3} concentration at pH 2.7) occurring at slightly negative electric fields. The most striking aspect of 5-FC condensation is the pH dependence. In contrast to 5-IC and 5-BC, it does not form any compact film at neutral pH but only in a narrow, acidic pH range. Amongst the three derivatives, 5-FC has the lowest polarizability, whose value is close to that of cytosine (Table I). This latter does not condensate either at neutral pH and small electric fields, and we believe that dispersion forces are not a major contribution in the formation of ordered layers of 5-FC. However, it is worth noting that the pH range favourable to two-dimensional condensation of 5-FC (pH 2.1–4.1) spans its pK_{a1} value. This fact strongly suggest that the film is composed of both

TABLE I

Selected properties of cytosine and its halogen derivatives. pK_{a1} and pK_{a2} are the first and second acid dissociation constant of the derivatives

C-5 group	H	F	Br	I
pK_{a1}	4.30[39]	2.55[39]	2.70[39]	2.95[39]
pK_{a1}	12.20[40]	10.87[40]	10.33[40]	10.59[41]
Dipole moment, D	8.83[42]	6.01[42]	5.51[42]	5.68[42]
Polarizability, $4\pi\varepsilon_0^3$	13.00[38]	12.97[38]	16.00[38]	

protonated and neutral molecules and stabilised by hydrogen bonding. Indeed, H-bonding in hemiprotonated cytosine dimers is well-established both experimentally[35,36] and theoretically[37], and has been advocated as an important factor for the 2D condensation of cytosine[21,22]. When the pH is far away from the pK_{a1} value, the ratio of protonated to neutral 5-FC molecules significantly deviates from unity, preventing the formation of hemiprotonated dimers and leading to the disappearance of the pit.

The most polarisable derivative studied here is obviously 5-IC. Dispersion forces are thus expected to be significant, while the H-bonding ability of the neutral derivative tend to decrease with the size of the halogen substituent[38]. Therefore, the 2D condensation of 5-IC can be assumed to arise mainly from dispersion interactions, highly favourable at neutral pH where 5-IC is uncharged, and resulting in a high critical temperature. At low pH, neither hydrogen bonding nor dispersion are able to overcome the electrostatic repulsions between positively charged molecules. No indication is found for the formation of hemiprotonated dimers, probably due to the weak hydrogen bonding ability of 5-IC. At high pH values, electrostatic repulsions between negatively charged 5-IC certainly exists. Two effects can however explain the existence of diverse condensed layers at pH 11. First, anionic 5-IC is expected to be more polarizable than cationic 5-IC, so dispersion forces might still be sufficient to stabilise the layer, together with the co-adsorption of cations from the electrolyte. The second effect, already mentioned earlier, is that the solubility of 5-IC significantly increases at this pH, opening the way to a concentration driven condensation. Determining the exact nature of the different films formed at pH 11 in the presence of high concentrations of surfactant would require more investigations, but we can nevertheless safely attribute their existence to the high polarizability of 5-IC.

In light of the above discussion, the third derivative, 5-BC, can be seen as an intermediate case between 5-FC and 5-IC, not only in term of polarizability value but also of adsorption behaviour. At pH where 5-BC is uncharged, it behaves in a similar way as 5-IC thanks to its pretty high polarizability and, possibly, hydrogen bonding. The critical temperature is nevertheless smaller than in the case of 5-IC, matching the trend noted in the polarizability values. In contrast to 5-IC, a pronounced destabilisation of the condensed layer is observed at pH 11, due to electrostatic repulsions which are not sufficiently compensated by dispersion interactions. At low pH, 5-BC is less destabilised than 5-IC, probably because of its higher H-bonding ability. Although there is no evidence of the formation of hemiprotonated dimers, the growth of the first pit towards the negative potentials could suggest the implication of positively charged species.

While structurally very close, the three derivatives used in this study exhibit markedly distinct adsorption behaviours. We have shown that 2D condensation of these compounds occurs in experimental conditions favouring strong intermolecular attractions. Both dispersion forces and H-bonding should be considered, as well as electrostatic interactions. The relative amplitude of each contribution strongly depends on the pH.

Further works on 2D condensation of different nucleic acid bases and/or components could provide quantitative thermodynamic analysis, kinetics of 2D condensed film formation, use of different spectroscopical methods[43] (which could provide direct information of molecules orientation and prove electrochemical findings) and involving of new electrode materials, as for example single crystal surfaces.

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