Biophysical Chemistry 9 (1979) 355–367

INTERFACIAL BEHAVIOR OF URACIL DERIVATIVES

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Received 19 October 1978

The adsorption of a number of methylated uracil derivatives and of 5-fluorouracil has been studied by surface electrochemical methods at a mercury electrode. All derivatives exhibit an initial or dilute adsorption region where they are adsorbed flat on the electrode surface and are bound by π-electron overlap with the electrode. Uracil, thymine, 1,5-dimethyluracil, 5,6-dimethyluracil, 1,5,6-trimethyluracil and 5-fluorouracil undergo a surface reorientation from the initial flat orientation to a perpendicular orientation. This reorientation process occurs at quite characteristic potentials and bulk-solution activities for each compound. An unsubstituted N(3)-H group is an absolute requirement for a uracil derivative to be capable of adopting the perpendicular surface stance. In the perpendicular orientation the uracil derivative appears to be bound to the electrode primarily via a N(3)–H––(–) electrode bond although a similar but weaker hydrogen bond can be formed via the N(1)-H group for certain compounds.

1. Introduction

A recent report from this laboratory [1] presented results of the adsorption and related interfacial behavior or uracil, 1-, 3- and 5-methyluracil and 1,3- and 1,5-dimethyluracil at a mercury electrode-aqueous electrolyte solution interface. It was noted that all of these uracil derivatives exhibited an initial adsorption region where the electrode area occupied by one uracil derivative at monolayer surface coverage, 60–70 Å^2, was close to that expected if the molecules were adsorbed flat on the electrode surface, i.e., with the plane of ring atoms parallel to the electrode surface. Uracil, thymine and 1,5-dimethyluracil exhibited a second adsorption region where they appeared to rearrange on the surface and adopt a perpendicular orientation and occupy about 40 Å^2 per molecule. The evidence obtained indicated that methylation at the N(3) position destroyed the ability of uracil to adopt a perpendicular surface orientation. Several factors were invoked to explain the ability of uracil and some of its derivatives to adopt a perpendicular surface stance including the availability of hydrogens at N(3) and N(1) as binding sites, intermolecular stacking interactions when the molecules are in the perpendicular orientation, and the strength of the interaction between the molecule and the electrode surface when in the initial, flat surface orientation [1]. Because of the limited number of uracil derivatives examined these initial interpretations were somewhat speculative. In order further to probe the adsorption of uracil derivatives and the factors which govern the ability of such compounds to exhibit the flat to perpendicular surface reorientation process our earlier investigations have been extended to include all methylated derivatives of uracil.

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2. Experimental

2.1. Chemicals

6-Methyluracil was obtained from Vega-Fox, 5-fluorouracil from Calbiochem. Other methylated uracil derivatives were prepared from procedures described in the literature; 1,6-dimethyluracil (Lacey [2]), 3,5-dimethyluracil (Marx et al. [3] and Naito

et al. [4]), 3,6-dimethyluracil (Ma and Zuellner [5]), 5,6-dimethyluracil (Hull et al. [6]), 1,3,5-trimethyluracil (Wittenberg [7]), 1,3,6-trimethyluracil (Pfeiderer and Mosthaf [8]), 1,5,6-trimethyluracil (Senda et al. [9] and Chi and Kao [10]), 3,5,6-trimethyluracil (Draminski and Fiszer [11]), and 1,3,5,6-tetramethyluracil (Pfeiderer and Mosthaf [8]). The procedures described in these sources were often modified somewhat. Details of these modifications and the procedures utilized are presented in detail elsewhere [12].

All differential capacitance and electrocapillary measurements, with the exception of those for 5-fluorouracil, were performed on solutions of the uracil derivative dissolved in a 0.5 *M* sodium fluoride plus 0.01 *M* Na_2HPO_4 buffer solution of pH 8.0. Studies on 5-fluorouracil utilized a phosphate buffer pH 7.0 (10.37 g NaH_2PO_4 and 19.61 g Na_2HPO_4 dissolved in 1 ℓ of deionized water). This buffer was used to insure that 5-fluorouracil ($pK_a = 8$ [13]) was studied in its neutral form.

A stock solution of the uracil derivative was prepared at a concentration close to its saturation value in the buffer solution. Test solutions were prepared by appropriate dilution of this stock solution with the pure buffer solution. Test solutions were deaerated with water-saturated nitrogen before study.

2.2. Differential capacitance measurements

Differential capacitance measurements were obtained by a phase-selective a.c polarographic method described in earlier reports [14–17]. All uracil derivatives exhibited both d.c. and a.c. equilibrium [1] at all applied potentials and concentrations employed at times between 1 and >5s. For convenience, all capacitance measurements were made with the drop-time of the dropping mercury electrode (DME) controlled at 2.00s. The drop area of the DME at the time the a.c. current was sampled was 0.0160 cm^2.

2.3. Maximum bubble pressure measurements

The maximum bubble pressure technique was used to measure the interfacial tension of a mercury electrode at potentials and bulk solution concentrations where various uracil derivatives exhibited a capacitance pit. A siliconized j-shaped capillary was used having a radius at its tip of *0.00377* cm. The apparatus employed has been described elsewhere [16,17].

The cells used for differential capacitance and maximum bubble pressure measurements were maintained at 25 ± 1°C. All potentials are reported versus the saturated calomel electrode (SCE) at 25°C.

2.4. Activity measurements

Values of the self-association (stacking) constant for 1,3,5- and 1,3,6-trimethyluracil and 1,3,5,6-tetramethyluracil were determined by a partition method similar to that employed by Guttman and Higuchi [18]. Varying volumes of the stock solution of the uracil derivative (in 0.5 *M* NaF + 0.01 *M* Na_2HPO_4, pH 8.0) were transferred into a 50-ml flask. Sufficient buffer was then added to dilute the uracil solution to 20.0 ml. Then, 20.0 ml of spectrophotometric grade *n*-heptane (Fisher) was transferred into each flask. The flasks were stoppered tightly, agitated for 5 min and then placed in a constant temperature bath at 25°C for about 12–14 h. An aliquot of the organic phase was withdrawn and appropriately diluted to allow its absorbance to be measured (Cary Model 118 Spectrophotometer) using 1.0 cm quartz cells. The wavelengths used for such measurements were 266 nm for 1,3,5-trimethyluracil and 1,3,5,6-tetramethyluracil and 260 nm for 1,3,6-trimethyluracil. Plots of the absorbance of the heptane layer, A, versus the bulk concentration of the uracil derivative in the buffer solution, C_{Aq}, were non-linear at high values of C_{Aq} indicating self-association of the uracil derivatives.

Further treatment of the A versus C_{Aq} data relied on the assumption that although the uracil derivative present in the aqueous phase is composed at monomers, dimers and oligomers of the compound, only the monomeric species is present in the heptane layer [18]. Individual solute species are assumed to obey Henry's law. Examination of the self-association behavior of a number of purines and pyrimidines suggests that the data are incompatible with a model which assumes that only dimers form [19–21]. Most results are consistent with a model in which monomers, dimers and oligomers form and in which the equilibrium constant for all of these steps is the same [22]. Under such conditions the total solute concentration, [C], is given by eq. (1),

$$[C] = C_M + 2KC_M^2 + 3K^2C_M^3 + \ldots \quad (1)$$

where C_M is the monomer concentration in the aqueous solution, and K is the self-association constant. Such an equation can be shown [23] to converge to

$$[C] = C_M/(1 - KC_M)^2. \quad (2)$$

Since the absorbance in the organic phase, A, is assumed to be due to monomer only, it must be proportional to monomer concentration in the aqueous phase, i.e.,

$$A = kC_M \quad (3)$$

and hence,

$$[C] = (A/k)/\{1 - (K/k)A\}^2. \quad (4)$$

The value of K was obtained by a non-linear least squares method which fits values of A at various bulk solution concentrations [C]. This was done by solving eq. (4) to obtain calculated values of A using trial values of the two parameters k and K. The non-linear least squares procedure [24] computes optimum values of k and K and standard errors in these parameters. Activity values, i.e. C_M, may be calculated from eq. (2).

3. Results

3.1. Activity determinations

Although most of our previous adsorption reports have employed bulk solution concentrations rather than activities it is well known that the tendency for self-association of pyrimidines is enhanced by substitution of methyl groups [25]. Accordingly, measurements of the self-association constants for some of the more highly methylated derivatives were carried out to ascertain whether activity effects could significantly influence the interpretation of adsorption data.

Values of the equilibrium self-association constant for 1,3,5- and 1,3,6-trimethyluracil and 1,3,5,6-tetramethyluracil in 0.5 *M* NaF plus 0.01 *M* Na_2HPO_4 buffer were measured by a partition method described in detail in section 2. Typical values of the self-association constant obtained at 25°C for the latter three compounds are presented in table 1. During the course of this work Pleisiewicz et al. [26] reported values of the self-association constant for these compounds measured by vapor pressure osmometry but in pure unbuffered aqueous solution at 25°C. The values reported by these workers are in excellent agreement with those reported in table 1. The values obtained for the self-association constants for the three uracil derivatives reported in table 1 and by Pleisiewicz et al. [26] for these and other derivatives indicate that at the concentrations used in adsorption studies concentration and activity are virtually identical. Accordingly, bulk solution concentrations of all uracil derivatives were taken as equal to activity values.

Table 1
Equilibrium constants for the self-association of methylated uracil derivative at pH 8.0 [a] at 25°C

Compound	K/M^{-1}
1,3,5-Trimethyluracil	1.27 ± 0.08
1,3,6-Trimethyluracil	1.0 ± 0.1
1,3,5,6-Tetramethyluracil	2.5 ± 0.2

[a] Buffer solution: 0.5 *M* NaF plus 0.01 *M* Na_2HPO_4, pH 8.0.

3.2. Differential capacitance measurements

A typical set of differential capacitance versus potential (C versus E) curves for some di- and trimethylated uracils and 1,3,5,6-tetramethyluracil are presented in fig. 1. Only one compound shown in fig. 1, 5,6-dimethyluracil, exhibits the well-defined capacitance pit centered at about −0.65 V. Two other compounds, 1,5,6-trimethyluracil and 5-fluorouracil exhibit a similar capacitance pit. Previous work [1,16] has shown that uracil, thymine (5-methyluracil) and 1,5-dimethyluracil also give rise to a capacitance pit. In the case of these six uracil derivatives it is clear that there are two distinct adsorption regions. Thus, similar to all other uracil derivatives, at low bulk solution activities there is a general depression of capacitance, compared to the pure background electrolyte solution, between about −0.2 V and −0.8 V followed by a broad adsorption/desorption peak at more negative potentials. The range of concentrations where this behavior is observed is called the initial or dilute adsorption region. In the case of uracil, thymine, 1,5-dimethyluracil, 5,6-dimethyluracil, 1,5,6-trimethyl-

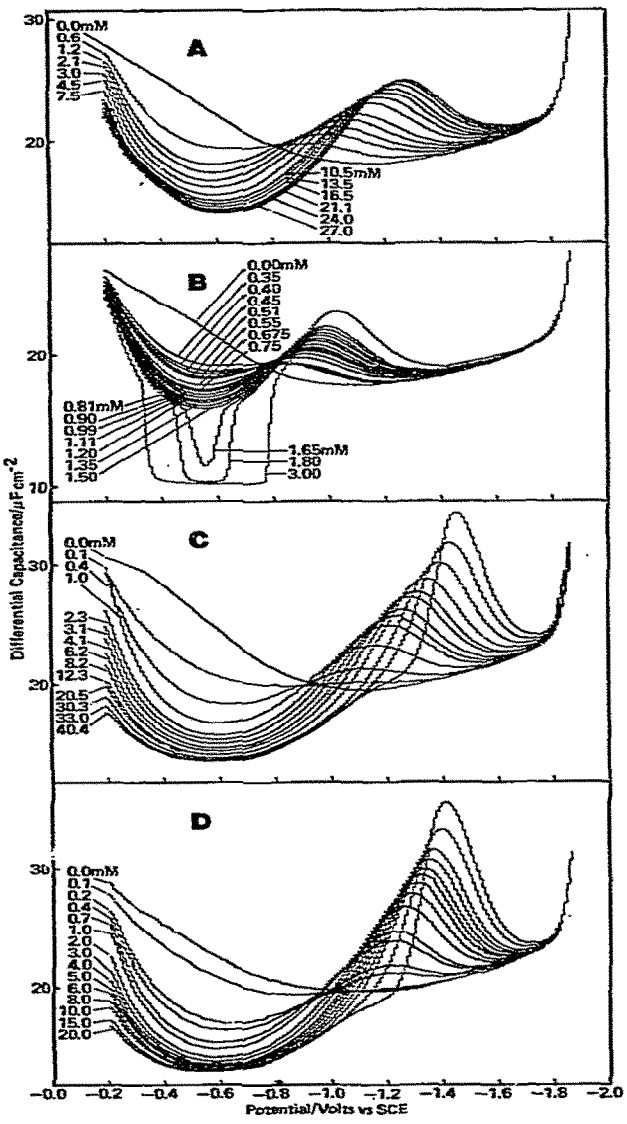

Fig. 1. Typical differential capacitance versus potential curves for (A) 3,5-dimethyluracil, (B) 5,6-dimethyluracil, (C) 1,3,5-trimethyluracil and (D) 1,3,5,6-tetramethyluracil in 0.5 *M* NaF with 0.01 *M* Na_2HPO_4 buffer pH 8.0. All curves were obtained at a frequency of 100 Hz and an amplitude of 10 mV peak-to-peak.

Table 2
Range of potentials over which adsorption isotherms of uracil derivatives are congruent with respect to potential [a]

Compound [b]	Potential range over which congruence exists/Volts versus SCE
6-Methyluracil	−0.6 V to ≥−1.1 V
1,6-Dimethyluracil	≤−0.5 V to ≥−1.0 V
3,5-Dimethyluracil	≤−0.5 V to ≥−1.0 V
3,6-Dimethyluracil	≤−0.5 V to ≥−1.0 V
5,6-Dimethyluracil	≤−0.5 V to ≥−1.0 V
1,3,5-Trimethyluracil	−0.9 V to ≥−1.4 V
1,3,6-Trimethyluracil	−0.9 V to ≥−1.4 V
1,5,6-Trimethyluracil	−0.8 V to ≥−1.2 V
3,5,6-Trimethyluracil	not congruent
1,3,5,6-Tetramethyluracil	−0.8 V to ≥−1.3 V
5-Fluoroaracil	≤−0.4 V to ≥−0.9 V

[a] Determined from ΓRT versus q plots.
[b] Data on the other uracil derivatives may be found in table 1 of ref. [1].

uracil and 5-fluorouracil the range of concentrations and potentials where the sharply defined discontinuity in C versus E curves occurs (see fig. 1B) is the second or capacitance pit region of adsorption.

In the dilute adsorption region all compounds gave C versus E curves which coincided with the background electrolyte curve at about −1.6 V or more negative. Accordingly, interfacial tension, γ, data were obtained by use of the double back-integration method of Grahame et al. [27]. The details of the procedure employed have been described in detail elsewhere [1,14–17]. Using such a method, values of the surface spreading pressure, π(dyne cm^{-1}) were obtained as a function of the uracil derivative activity, a, and electrode potential, E, using the equation

$$\pi(E) = \gamma_w(E) - \gamma(E), \qquad (9)$$

where γ_w is the value of γ for the pure supporting electrolyte solution at $a = 0$. For each uracil derivative studied, with the single exception of 3,5,6-trimethyluracil, plots of π versus ln a at many different potentials were superimposable by abscissa translation. Such superimposability of π versus ln a curves has been used to prove congruence of electrosorption isotherms with respect to potential. However, as Parsons [28] has noted, the sensitivity of this procedure is perhaps suspect. Accordingly, congruence was further

Table 3
Parameters of the generalized Frumkin equation for uracil derivatives determined from capacitance measurements at pH 8 [a] in the dilute adsorption region

Compound	α	$B_0 \times 10^{-3}$ ($\ell \cdot$ mole^{-1})	ΔG^0 [c] (cal)	C' (μF cm^{-2})	E_N (Volts versus SCE)	Area per molecule (A)	$\Gamma_m \times 10^{10}$ (mole cm^{-2})	rmsd (dynes cm^{-1})
Uracil [b]	0.45 ± 0.08	0.158 ± 0.005	−3000	14.3 ± 0.4	−0.474 ± 0.003	64 ± 3	2.6 ± 0.1	0.156
1-Methyluracil [b]	−0.42 ± 0.09	0.66 ± 0.02	−3800	12.4 ± 0.3	−0.386 ± 0.006	71 ± 2	2.34 ± 0.07	0.199
3-Methyluracil [b]	−0.6 ± 0.1	0.83 ± 0.04	−3900	11.7 ± 0.4	−0.40 ± 0.01	70 ± 3	2.4 ± 0.1	0.188
5-Methyluracil [b]	0.21 ± 0.04	0.49 ± 0.01	−3700	10.7 ± 0.3	−0.411 ± 0.003	57 ± 2	2.9 ± 0.1	0.088
6-Methyluracil	0.0 ± 0.2	0.56 ± 0.05	−3700	12.1 ± 0.7	−0.41 ± 0.03	72 ± 3	2.3 ± 0.1	0.359
1,3-Dimethyluracil [b]	−0.42 ± 0.06	1.71 ± 0.04	−4400	11.1 ± 0.2	−0.309 ± 0.006	75 ± 1	2.21 ± 0.03	0.148
1,5-Dimethyluracil [b]	0.20 ± 0.08	1.12 ± 0.04	−4200	11.2 ± 0.4	−0.325 ± 0.009	71 ± 3	2.3 ± 0.1	0.105
1,6-Dimethyluracil	−0.15 ± 0.07	0.57 ± 0.02	−3800	12.4 ± 0.3	−0.374 ± 0.007	65 ± 1	2.54 ± 0.04	0.214
3,5-Dimethyluracil	0.11 ± 0.09	0.93 ± 0.04	−4000	12.6 ± 0.3	−0.359 ± 0.008	88 ± 2	1.92 ± 0.04	0.888
3,6-Dimethyluracil	−0.42 ± 0.07	1.34 ± 0.04	−4300	12.9 ± 0.2	−0.388 ± 0.004	81 ± 1	2.05 ± 0.03	0.177
5,6-Dimethyluracil	1.14 ± 0.09	0.71 ± 0.05	−3900	13.6 ± 0.8	−0.37 ± 0.01	80 ± 7	2.1 ± 0.2	0.198
1,3,5-Trimethyluracil	0.10 ± 0.09	3.8 ± 0.6	−4900	12.3 ± 0.4	−0.34 ± 0.02	91 ± 2	1.82 ± 0.04	0.167
1,3,6-Trimethyluracil	−0.17 ± 0.07	3.6 ± 0.4	−4800	11.7 ± 0.4	−0.32 ± 0.02	91 ± 2	1.83 ± 0.04	0.138
1,5,6-Trimethyluracil	1.17 ± 0.06	1.0 ± 0.2	−4100	8.0 ± 2	+0.1 ± 0.2	89 ± 18	1.90 ± 0.4	0.077
3,5,6-Trimethyluracil						85 [d]		
1,3,5,6-Tetramethyluracil	0.0 ± 0.1	2.8 ± 0.4	−4700	8.8 ± 0.2	−0.15 ± 0.06	75 ± 3	2.22 ± 0.09	0.247
5-Fluorouracil	0.21 ± 0.06	0.139 ± 0.003	−2900	13.4 ± 0.3	−0.417 ± 0.004	74 ± 2	2.23 ± 0.06	0.172

[a] 0.5 *M* NaF plus 0.01 *M* Na_2HPO_4. [b] From ref. [1]. [c] The value of the free energy of adsorption at the ECM potential (−0.433 V) for the pure supporting electrolyte, $\Delta G^0 = -RT \ln B_0$. [d] Calculated from the limiting slope of π versus ln a plots.

tested by preparing plots of ΓRT calculated by analytical differentiation of fixed potential π versus $\ln a$ curves (as described in an earlier report [1]), versus electrode charge, q. Linear ΓRT versus q plots were obtained over large ranges of electrode potential for all uracil derivatives except 3,5,6-trimethyluracil indicating congruence of electrosorption isotherms with respect to potential [1]. The ranges of potentials over which electrosorption isotherms were congruent with respect to potential are shown in table 2.

In view of the fact that in the dilute adsorption region the adsorption isotherms for most uracils are congruent with respect to potential, π, E and a data were fitted to the generalized form of the Frumkin isotherm equation (eq. 10). In this equation Γ_m is the limiting surface excess of solute at full monolayer

$$\theta/(1-\theta) = B_0 a \exp\{2\alpha\theta\} \exp\{-\phi/\Gamma_m RT\} \qquad (10)$$

coverage (moles cm^{-2}), α is the lateral attraction coefficient and B_0 is a constant related to the free energy of adsorption at the ECM potential for the pure supporting electrolyte solution ($\Delta G^0 = -RT \ln B_0$). The function

$$\phi = [\gamma_w(0) - \gamma_w(E)] + C'EE_N - C'E^2/2 \qquad (11)$$

is evaluated in terms of E, the potential relative to the ECM potential for the pure supporting electrolyte solution, E_N the ECM for the mercury solution interface when $\theta = 1$, and C' the capacitance at $\theta = 1$ (assumed to be constant [29]), and the interfacial tension for the pure supporting electrolyte solution γ_w.

A non-linear least-squares computational method was used to determine the values of α, B_0, Γ_m, E_N and C' using experimental π, E and a data [1,14–17]. Results of such analysis are presented in table 3. Of the seventeen compounds reported in table 3, data for five compounds were obtained from an earlier study [1] but are included for comparative purposes.

In the case of 3,5,6-trimethyluracil the electrosorption behavior was not congruent with respect to potential (vide supra). Accordingly, a detailed analysis of its adsorption behavior was not possible employing the earlier methods. The Γ_m and area occupied per molecule on the electrode surface reported in table 3 were obtained graphically by measuring the limiting slope of π versus $\ln a$ curves at different potentials.

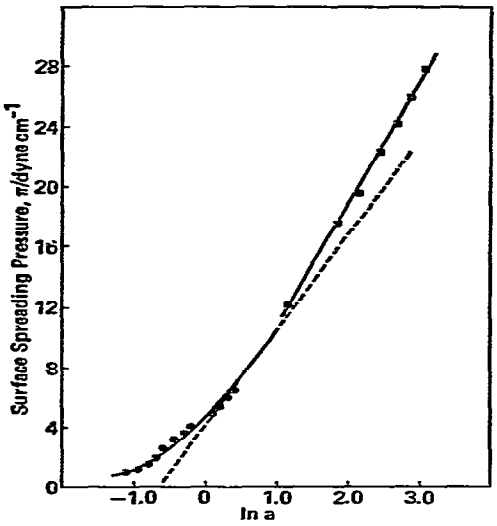

Fig. 2. Surface spreading pressure, π, versus $\ln a$ plot for 5,6-dimethyluracil in 0.5 M NaF plus 0.01 M Na_2HPO_4, pH 8.0. Solid circles (●) refer to experimental points obtained at −0.5 V in the dilute adsorption region by analysis of differential capacitance data. Solid squares (■) refer to experimental points obtained at −0.5 V at concentrations where the capacitance pit is observed using the maximum bubble pressure method. Line through solid circles is the best composite π versus $\ln a$ fit.

3.3. Maximum bubble pressure measurements

The method of double back-integration of C versus E curves cannot easily be employed to calculate interfacial tension and hence π values in the potential regions where capacitance pits occur. Therefore, γ and π values in the capacitance pit regions were obtained directly using maximum bubble pressure measurements. Only three of the uracil derivatives studied exhibited a capacitance pit: 5,6-dimethyluracil, 1,5,6-trimethyluracil and 5-fluorouracil. However, interfacial tension measurements were taken only for the first two compounds. This was so because 5-fluorouracil exhibited a capacitance pit only at concentrations close to saturation and hence it was not possible to obtain sufficient π and a data to allow Γ_m values to be calculated. 5,6-Dimethyluracil exhibited the capacitance pit at concentrations greater than 1.6 mM. In the case of 1,5,6-trimethyluracil concentrations greater than 0.3 mM were required.

Table 4
Areas occupied per molecule at maximum surface coverage in the dilute and capacitance pit regions for various uracil derivatives at pH 8 [a]

Compound	Area per molecule ($Å^2$)	
	in the dilute adsorption region	in the capacitance pit region
Uracil [b]	63 ± 3	40
5-Methyluracil [b]	66 ± 2	39
1,5-Dimethyluracil [b]	70 ± 3	43
5,6-Dimethyluracil	80 ± 7	52 ± 1
1,5,6-Trimethyluracil	89 ± 18	48 ± 7

[a] 0.5 *M* NaF plus 0.01 *M* Na_2HPO_4 buffer.
[b] From ref. [1].

Using π values obtained from *C* versus *E* curves in the dilute adsorption region and from maximum bubble pressure measurements in the capacitance pit region it was found that composite plots of π versus ln *a* exhibited a sharp change in slope at the bulk solution activity where the capacitance pit was noted (fig. 2). The dotted line in fig. 2 represents the limiting slope for the π versus ln *a* plot in the dilute adsorption region. Clearly, at concentrations where the capacitance pit is observed the slope of the π versus ln *a* plot becomes considerably steeper. The more steeply sloping region of the π versus ln *a* curve corresponds to larger Γ_m values and hence to smaller areas occupied by the uracil derivative in the capacitance pit region compared to the dilute region. The areas occupied by various uracil derivatives at complete monolayer coverage in the dilute and capacitance pit region are shown in table 4.

3.4. Interpretation of adsorption results

In the dilute adsorption region the observed electrosorption behavior of all uracil derivatives, with the single exception of 3,5,6-trimethyluracil, fit well to the Frumkin adsorption model (table 3). The attraction coefficients, α, are generally quite small indicating relatively weak intermolecular interactions between the adsorbed organic molecules. On the other hand, the standard free energy of adsorption of the uracils at the ECM potential for the pure supporting electrolyte solution (–0.433 V) increases in an approximately systematic fashion with increasing substitution of uracil with methyl groups. Thus, for unsubstituted uracil ΔG^0 = –3000 cal, monosubstituted uracils have an average ΔG^0 of –3775 ± 125 cal, disubstituted uracils –4100 ± 300 cal and trisubstituted uracils –4600 ± 500 cal. In other words, increasing substitution of uracil leads to an enhanced adsorption of the compound in the dilute adsorption region.

The areas occupied by the uracil derivatives also generally increase with methylation. Thus, the monomethylated derivatives occupy an average area of 69 ± 12 $Å^2$, the dimethylated derivatives 77 ± 12 $Å^2$ and the trimethylated derivatives 86 ± 11 $Å^2$. These should be compared to the value of 64 ± 3 $Å^2$ observed for unsubstituted uracil.

In order to decide the most probable surface orientation of uracil derivatives based on these latter areas, projected areas were calculated based on X-ray crystallographic data on uracil [30], thymine [31] and 1,5-dimethyluracil [32]. Such areas were calculated from projections of the molecule in a flat surface orientation using the ORTEP program of Johnson [33] which uses crystal structure data, i.e., unit cell parameters, unit cell type and atomic coordinates, as a means of projecting the molecule in any desired orientations. Using van der Waals radii for C, H, N and O of 1.65 Å, 1.2 Å, 1.5 Å and 1.4 Å, respectively, the projected areas for uracil, thymine and 1,5-dimethyluracil were found to be 51 $Å^2$, 63 $Å^2$ and 67 $Å^2$, respectively (fig. 3A, B, C). Unfortunately, crystal structure data are not available for other di-, tri- or tetramethylated uracil derivatives. However, estimations of the approximate areas these molecules would occupy in a flat surface orientation were made by utilizing the projected area for 1,5-dimethyluracil (fig. 3C) and assuming that all other methyl groups have bond distances and angles similar to those of the methyl groups of the latter compound. An example of such an approximation is shown in fig. 3D for 1,3,5-trimethyluracil (74 $Å^2$) and in fig. 3E for 1,3,5,6-tetramethyluracil (78 $Å^2$). In all cases these projected areas are in quite reasonable accord with those measured experimentally for uracil derivatives in the dilute adsorption region (see earlier discussion and table 3) which strongly supports the view that in the dilute adsorption region all uracil derivatives are adsorbed in a flat orientation on the electrode surface.

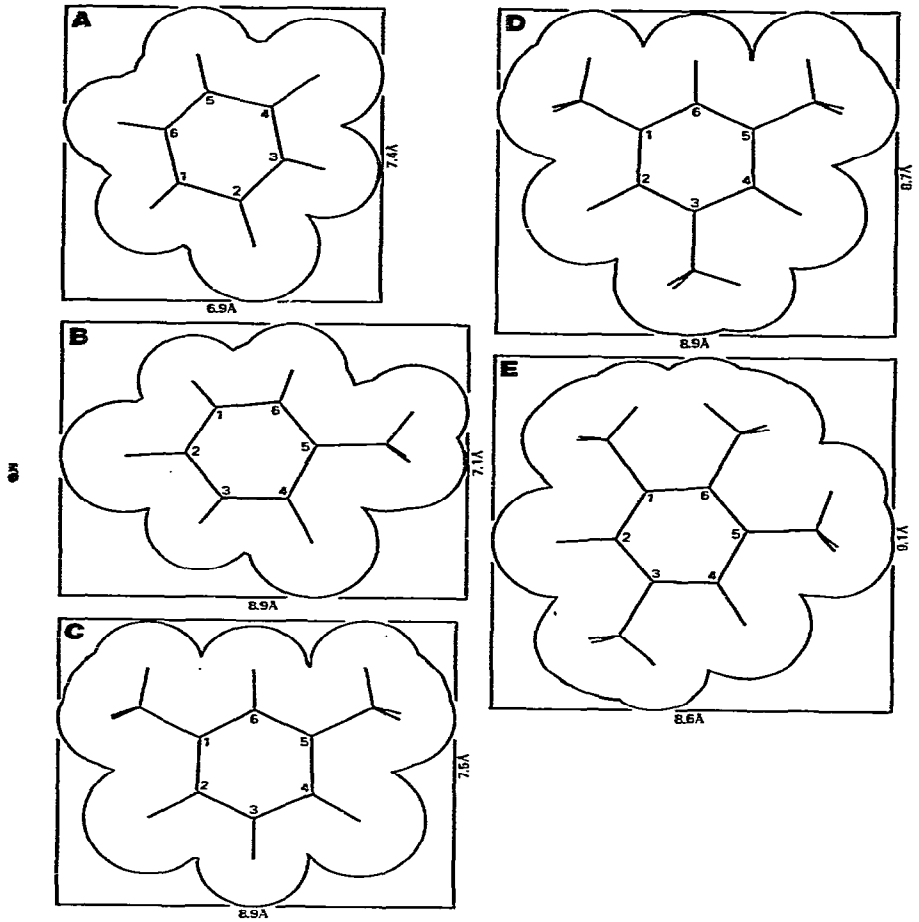

Fig. 3. Projected areas calculated for (A) uracil, (B) thymine, (C) 1,5-dimethyluracil, (D) 1,3,5-trimethyluracil and (E) 1,3,5,6-tetramethyluracil in a flat surface orientation (plane of ring atoms parallel to the electrode surface). See text for discussion.

The interaction of π-orbital electrons with the electrode surface is probably an important factor in binding uracil derivatives in the flat orientation in the dilute adsorption region. Such interactions have been shown to be important in the electrosorption of conjugated systems and aromatic compounds [35]. That π-orbital interactions with the electrode are important may be deduced by considering the effect of methyl substituents on the π-electron system of uracil. Uracil may be regarded as a π-deficient molecule [36,37] principally because of the electronegative nitrogen atoms which tend to withdraw electron density from the π-system of the ring. Thus, one would expect uracil to be relatively weakly adsorbed. Indeed, it is found experimentally that uracil has the smallest free energy of adsorption (—3000 cal, table 3 [1]) of all compounds studied with the exception of 5-fluorouracil (—2900 cal). The highly electronegative fluorine substituent would be expected to increase the π-deficient character of uracil and hence decrease the binding between the π-electron system and the electrode surface. Conversely, introduction of electron-releasing methyl groups into the uracil ring would act to make the molecule less electron-

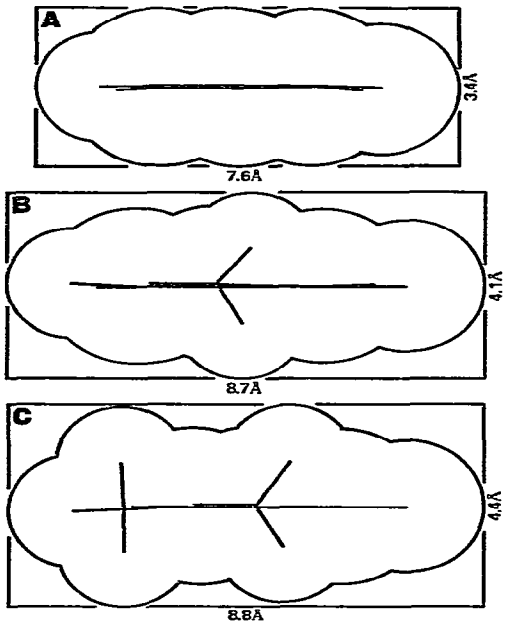

Fig. 4. Projected areas calculated for (A) uracil, (B) thymine, and (C) 1,5-dimethyluracil in a perpendicular surface orientation (plane of ring atoms perpendicular to the electrode surface). The areas for 5,6-dimethyluracil and 1,5,6-trimethyluracil were the same as for 1,5-dimethyluracil.

deficient, particularly when the methyl group is substituted at an electronegative nitrogen. Comparing monomethylated uracil derivatives (table 3) it is observed that 1- and 3-methyluracil have the largest negative ΔG^0 values of all trimethylated uracil derivatives. Such observations strongly support the idea that in the flat orientation the major binding force occurs between the uracil π-electrons and the electrode surface. Other theoretical calculations of charge distribution in methylated uracils also support the latter conclusion [38]. Thus, such calculations indicate that the increased π-electron density created in the uracil ring by substitution at a nitrogen propagates over the N(1)-C(2)-N(3)-C(4) system, i.e. over a large segment of the molecule. On the other hand, methyl substitution at C(5) and/or C(6) gives increased π-electron density only in the C(5)=C(6) double bond.

At concentrations and potentials where 5,6-dimethyluracil and 1,5,6-trimethyluracil give rise to the capacitance pit the area occupied by these molecules, assuming monolayer surface coverage, decreases by about 40 per cent. Similar behavior was observed previously in the case of uracil, thymine and 1,5-dimethyluracil [1]. Calculated projections of the areas these molecules would occupy in a perpendicular surface orientation (fig. 4) indicate that the area occupied by uracil should be 26 Å^2, thyming 35 Å^2, 1,5-, 1,6-dimethyluracil and 1,5,6-trimethyluracil 39 Å^2. These areas are again in close agreement with those measured experimentally in the capacitance pit region (table 4) and support the view that in the latter region the uracil derivatives are adsorbed on the electrode surface in a perpendicular orientation. It should be noted that in the capacitance pit region the adsorbed perpendicular uracil molecules must be in such close proximity to each other that very extensive intermolecular stacking interactions must take place on the electrode surface, very similar to these interactions which occur between adjacent purine and pyrimidine bases in nucleic acids [34]. A further justification for this effect comes from the fact that a systematic and large decrease in the bulk solution activity required to cause formation of the perpendicular, stacked layer is observed with increasing methylation of the uracil ring. Thus, a bulk solution activity of about 21 mM is required to observe the surface reorientation process (capacitance pit) for uracil, while an activity of 11 mM is required in the case of thymine, 1.6 mM for 5,6-dimethyluracil and 0.3 mM for 1,5,6-trimethyluracil. Methylation of the uracil ring is known to enhance stacking interactions [26] and hence would be expected to facilitate formation of the perpendicular, stacked layer of uracil derivative molecules on the electrode surface. The electron-withdrawing fluorine atom in 5-fluorouracil would, conversely, be expected to decrease the tendency for stacking interactions and this is observed by the fact that the capacitance pit does not occur until the bulk solution activity reaches 100 mM. The first appearance of the capacitance pit in C versus E curves and hence the occurrence of the flat-to-perpendicular surface reorientation of uracil derivatives occurs only when the electrode has a surface coverage of molecules in the flat orientation in excess of 90 percent. In other words, monolayer surface saturation in the flat orientation must be approached

before the reorientation of adsorbate molecules can occur.

It is also noticeable from the results presented in tables 3 and 4 that methylation of uracil at the N(3) position always destroys the ability of a uracil derivative to exhibit the capacitance pit, i.e. flat-to-perpendicular surface reorientation. This, therefore, strongly supports the conclusion that an unsubstituted N(3) position is essential for a uracil derivative to be capable of exhibiting a perpendicular surface orientation. However, it may be noted (table 4) that 1-methyluracil, 1,6-dimethyluracil and 6-methyluracil are also unable to exhibit a capacitance pit. However, 1,5-dimethyluracil and 1,5,6-trimethyluracil can exhibit a capacitance pit, i.e. a perpendicularly orientated surface layer. This behavior reinforces an earlier suggestion [1] from this laboratory that not only is the hydrogen at N(3) capable of binding uracil to the electrode but also the hydrogen at N(1). However, the fact that no uracil derivative methylated at N(3) can give a perpendicular layer but several derivatives methylated at N(1) can give this layer indicates that the N(3)-H is a strong perpendicular binding site whereas N(1)-H is a significantly weaker perpendicular binding site.

In order to explain the ability of a uracil derivative to adopt a perpendicular surface orientation it is clear that the first and absolute requirement is that the N(3) position i.e. the strong perpendicular binding site, must be unsubstituted. However, three other factors must be considered: (a) the availability of the weaker N(1) binding site, (b) the effects of methylation on intermolecular stacking interactions between adjacent perpendicular uracil residues and (c) the steric effect of methylation on the ability of the N(1)-H group to effectively bind to the electrode surface. The latter three effects must be considered in order to rationalize the failure of 1-, and 6-methyluracil and 1,6-dimethyluracil to form a perpendicular surface layer.

It is believed that uracil is able to form a perpendicular layer because it has two perpendicular binding sites, N(1)-H and N(3)-H. The presence of these two binding sites is sufficient to anchor uracil in a perpendicular stance even though the stacking interactions between adjacent bases would be smaller than for methylated derivatives [26]. 1-Methyluracil cannot exhibit a perpendicular layer because it lacks a perpendicular binding site and the influence of a single methyl group at N(1) on stacking interactions must be quite small. The same argument must apply to 1,6-dimethyluracil when the N(1) and C(6) methyl substituents apparently do not enhance stacking interactions sufficiently to overcome the loss of the N(1)-H binding site. In the case of 6-methyluracil both perpendicular binding sites are available. However, molecular models reveal that the methyl substituent at C(6) should sterically hinder binding between N(1)-H and the electrodes surface. Hence 6-methyluracil should, and does, exhibit similar behavior to 1-methyluracil. In view of the fact that 1,5-dimethyluracil does exhibit a perpendicular surface layer but 1,6-dimethyluracil does not it must be concluded that the methyl group at the C(6) position has a significantly smaller effect on base-base stacking interactions than does the methyl group at the C(5) position. In other words 1,5-dimethyluracil exhibits a perpendicular layer because the loss of the weaker N(1)-H perpendicular binding site is more than compensated for by the presence of the methyl groups at N(1) and C(5) which enhance stacking interactions such that a perpendicular layer may be formed and stabilized. The only reason, therefore, that a similar explanation cannot be applied in the case of 1,6-dimethyluracil is if the C(6)-methyl group is far less effective in promoting base-base stacking interactions. Unfortunately there is no experimental evidence available concerning the relative effect of C(6) and C(5) methyl groups on base-base stacking interactions.

In summary, therefore, it appears that the N(3)-H group is essential for a uracil derivative to be capable of exhibiting a perpendicular surface orientation but that the N(1)-H group can also function as a weaker binding site. Binding of a uracil derivative by a N(3)-H--- electrode or N(1)-H--- electrode bond suggests an interaction resembling a hydrogen bond. Such a hydrogen bond would normally require that the electrode carry a negative charge. In the capacitance pit region it is necessary to determine the ECM potential in order to decide the sign of the charge carried by the electrode. This was done by considering γ, E, and σ data obtained in the capacitance pit region using the maximum bubble pressure method. Interfacial tensions are more difficult to determine in the pit region than in the dilute adsorption region. This is so because there is a tendency for mercury to "stick" inside the glass capillary. Nonetheless, reasonably consistent sets

Table 5
Comparison of calculated ECM potentials for uracil derivatives in the capacitance pit region and the optimum potential for capacitance pit formation [a]

Compound	ECM potential/ [b] Volt versus SCE	Optimum potential [c] for pit formation/ Volt versus SCE
Uracil	−0.43	−0.56
Thymine	−0.37	−0.61
1,5-Dimethyluracil	−0.43	−0.53
5,6-Dimethyluracil	−0.42	−0.56
1,5,6-Trimethyluracil	−0.17	−0.60

[a] In 0.5 *M* NaF + 0.01 *M* Na_2HPO_4 pH 8.0 buffer.
[b] Calculated by the method described in text.
[c] The potential at which the capacitance pit is first observed.

of γ versus E values at different concentrations could be obtained in the capacitance pit region for uracil, 5-methyluracil, 1,5- and 5,6-dimethyluracil and 1,5,6-trimethyluracil. In analyzing these data it was noted that the measured capacitance in the pit region is essentially independent of potential for a given uracil derivative. Therefore, it is possible to express the interfacial tension as a function of activity and potential as

$$\gamma = \gamma_{ECM(a=1\,mM)} - \Gamma_m RT \ln a - \tfrac{1}{2}C'(E - E_{ECM})^2, \quad (12)$$

where $\gamma_{ECM(a=1\,mM)}$ is the hypothetical maximum in interfacial tension for the perpendicularly oriented film (at $\theta = 1$ at the ECM potential for that film and at a solution activity of 1 m*M*), and C' is the capacitance of the perpendicularly oriented film. Data for five uracil derivatives have been fitted to eq. (12) by least squares analysis to obtain values of the four parameters $\gamma_{ECM(a=1\,mM)}$, Γ_m, E_{ECM} and C'. The values of C' determined by the four-parameter fitting procedure were ordinarily close to those observed in the pit region for each system. Thus, using the observed value of C' for each system as a known constant leads to very nearly the same values of the other parameters as those obtained in the analysis which treats C' as an adjustable parameter. Moreover, Γ_m values inferred for the film formed in the capacitance pit region using eq. (12) were in good agreement with values calculated from the slope of plots of π versus ln a, at constant potential, in the pit region. Table 5 lists the values of E_{ECM} inferred from the least squares analysis and the potentials at which the capacitance pit first forms at the lowest solution concentrations. The ECM potentials reported for the perpendicular uracil layer in table 5 are, in general, not very different from the ECM potentials for the flat uracil layer at full monolayer coverage reported in table 3 (i.e. E_N values).

Also shown in table 5 are the optimum potentials for formation of a perpendicular layer i.e. the potential at which capacitance pit formation is first noted. It is clear that the optimum potential for formation of the perpendicular layer generally lies 0.1 V to 0.2 V negative of the ECM potentials for the pure supporting electrolyte solution, the flat adsorbed layer of uracil (E_N, table 3) or for the electrode covered with a perpendicular monolayer. Thus, the optimum conditions for formation of a perpendicularly oriented layer seem to prevail when the electrode carries a relatively small negative charge. This fact seems to support the idea that the uracil derivatives are bound to the electrode through the N(3)-H--- electrode hydrogen bond or, in some instances and to a lesser extent, a N(1)-H--- electrode hydrogen bond with the electrode functioning as a moderately electronegative site. As noted earlier, formation of the perpendicular adsorbate layer is facilitated by substitution of electron-releasing methyl groups into uracil because of the resultant increase in intermolecular stacking interactions between adjacent bases.

It may be observed in C versus E curves of uracil derivatives which exhibit the capacitance pit (fig. 1B, C and fig. 1 of ref. [1]) at potentials both more positive and negative than the optimum potential for pit formation that the capacitance dramatically increases to a value characteristic of the flat oriented surface layer. This implies that on both the positive and negative side of the optimum potential the perpendicular uracil layer undergoes a catastrophic disruption and forms a partial flat layer. Although it is not possible at this stage to definitively explain this catastrophic breakdown of the perpendicular layer it seems reasonable to assume that at potentials sufficiently negative or positive of the optimum potential, water molecules begin to compete successfully with the uracil derivative for the electrode surface. Since the perpendicular layer of uracil derivatives are stabilized by highly co-

operative stacking interactions it is likely that displacement of even a few molecules by water would result in total collapse of the perpendicular layer.

3.5. Conclusions

The results reported in this and an earlier report [1] support a number of general conclusions regarding the adsorption and related interfacial behavior of uracil derivatives. First, all uracil derivatives studied exhibit an initial adsorption region where they are adsorbed flat on the electrode surface over a large range of potentials. The interaction between the adsorbed molecules and the electrode responsible for binding the adsorbate to the surface appears to be between the π-electron cloud and, presumably, the conduction band of the electrode. In effect a rather weak, non-specific bond anchors the uracil molecule to the electrode. The effect of methyl and fluoro substituents on the π-electron density and hence on the standard free energy of adsorption is in accord with the above type of interaction.

Uracil, and 5-fluorouracil and a number of methylated uracil derivatives undergo a surface rearrangement from the initial flat orientation to a perpendicular orientation. This reorientation process occurs at potentials characteristic of each compound. However, the optimum potential for the flat-to-perpendicular reorientation process always occurs at a negatively charged electrode. In addition, an unsubstituted N(3)-H function appears to be an absolute requirement for a uracil derivative to be capable of adopting the perpendicular surface layer. Accordingly, it seems reasonable to conclude that when in a perpendicular stance the uracil derivative forms, in effect, a hydrogen bond of the type N(3)-H--- (-) electrode. Since the N(1)-H group can apparently also function as a perpendicular binding site, although considerably less effectively than the N(3)-H group, a similar hydrogen bond may be formed between the N(1)-H group and the negatively charged electrode. Increasing methyl substitution of uracil increases the π-electron density of the uracil ring which in turn favors formation of the perpendicular layer as a result of enhanced intermolecular stacking interactions between adjacent bases. Conversely, a fluoro substitutent decreases the tendency to form the perpendicular layer because of a decrease in π-electron density.

At potentials sufficiently negative or positive of the optimum value for formation of the perpendicular layer a very sharply defined collapse of the latter layer occurs. This is apparently so because at such potentials water molecules can successfully compete with the uracil derivative for surface sites. Because of the highly cooperative nature of the perpendicular layer, replacement of even a few uracil molecules by water causes the entire perpendicular array to revert to a flat oriented layer.

The perpendicular layer of adsorbed uracil derivative exhibits properties at the electrode surface typical of such molecules in nucleic acids, i.e., they bind to the surface through their normal Watson-Crick hydrogen bonding site and undergo stabilization by means of stacking interactions.

Acknowledgement

The authors are pleased to acknowledge partial support for this work by the National Institutes of Health through Grant No. GM 21034-03. The support of the University of Oklahoma Research Council is also gratefully acknowledged.

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