

## 446 — ELECTROCHEMICAL EXAMINATION OF NUCLEOSIDE INTERACTION

### ADENOSINE—GUANOSINE AND CYTIDINE—GUANOSINE

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#### SUMMARY

The adsorption at the solution|mercury electrode interface of guanosine, adenosine and cytidine alone and in guanosine—adenosine and guanosine—cytidine mixtures was examined for pH 4.0 and 7.2 solutions at 25 and 40°C; the principal index used was the *a.c.* polarographic quadrature current component. All of the nucleosides are adsorbed at the 1.0 mM and lower concentrations examined. There is evidence at 25°C for:

- (a) the self-association of uncharged adsorbed guanosine;
- (b) formation at pH 4 of a 2 : 1 guanosine—adenosine complex, which is at least partially positively charged, is more stable than the self-associated guanosine and associates in the adsorbed state;
- (c) formation at pH 7.2 of a similar 2 : 1 but uncharged guanosine—adenosine complex;
- (d) formation of a probably positively charged 1 : 1 guanosine—cytidine complex, which adsorbs and associates at pH 4 and of a similar neutral complex at pH 7.2.

Association of the adsorbed species is not seen at 40°C. The controlling factor in determining the differential capacitance at the interface is generally the presence of the most surface-active species, guanosine.

#### INTRODUCTION

Numerous studies of the adsorption behavior of the individual nucleic acid bases and their nucleosides, as well as of more complex species, have been published in the past decade; many of these have involved the use of alternating current (*a.c.*) polarography. Typical are the studies by Vetterl, Valenta, Nürnberg, Christian, Dryhurst and their collaborators. Most germane to the present study are those on adenosine [1–6], cytidine [1,2,7] and guanosine [1,2], and Vetterl's seminal examinations of nucleosides [1,2]; the latter papers provide much of the basis for subsequent discussion of the *pits* or *wells* in capacitive current—potential plots. Vetterl's 1977 paper [8] provides useful background and references.

The present paper describes a study of the complexes formed by guanosine with adenosine and cytidine, which complexes are adsorbed at the aqueous solution|mercury interface where they can associate. The primary indicator

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used is the variation with potential of the quadrature current component seen on phase-selective *a.c.* polarography. In the absence of a faradaic process, the quadrature current component is proportional to the double-layer capacitance at the interface and, consequently, provides a readily obtainable index to the adsorption of species at the interface and to the variation in their nature.

Guanosine and adenosine are 9- $\beta$ -D-ribofuranosyl derivatives; cytidine is 3- $\alpha$ -ribosidocytosine. The generally seen order of surface activity is guanosine > adenosine > cytidine.

In order to minimize complications associated with reorientation of the adsorbed species from the dilute adsorption layer — characterized by the species being oriented parallel to the electrode surface — to an orientation perpendicular to the surface, as the nucleoside concentration increases [4,8,9], the concentrations of the nucleosides were kept at or below 1 mM. A minor advantage of working at such concentrations is that these are in the range involved in normal polarographic and voltammetric studies.

The present study will hopefully provide an extension of the use of phase-selective *a.c.* polarography for examining adsorption to the investigation of complexation, association and interfacial film formation at low concentrations of nucleic acid and other biologically significant species.

## EXPERIMENTAL

The pertinent theory of *a.c.* polarography and the isolation and measurement of the quadrature current component are described in the literature [10–13].

The  $pK_a$  values for the compounds examined (where (*b*) indicates a basic  $pK_a$ , i.e., one for proton addition, and (*a*) indicates acidic  $pK_a$ , i.e., one for loss of a proton) are as follows: adenine, 0–1 (*b*), 4.1 (*b*), 9.8 (*a*); adenosine, 3.5 (*b*), 12.5 (*a*); cytosine, 4.5 (*b*), 12.2 (*a*); cytidine, 4.2 (*b*), 12.5 (*a*); guanine < 0 (*b*), 3.2 (*b*), 9.6 (*a*), 12.4 (*a*); guanosine, 1.6 (*b*), 9.2 (*a*), 12.4 (*a*).

The pH values examined were selected on the basis that both adenosine and cytidine produce well-formed, diffusion-controlled dropping mercury electrode (d.m.e.) waves at pH 4.0, and that pH 7.2 is commonly used as a reference pH for biological phenomena and reactions.

### *Apparatus*

Electrochemical measurements were made with a Princeton Applied Research (PAR) Model 174 polarographic unit, a Model 174/50 *a.c.* polarographic analyzer interface and a Model 122 lock-in amplifier, and were recorded on a Hewlett—Packard (HP) Model 7005B X—Y recorder. The d.m.e. drop time was controlled, when so desired, by a PAR Model 172 drop timer and knocker. Potentials were monitored with a HP Model 3430A digital voltmeter.

The water-jacketed cell used was kept at 25 or 40°C. The working electrode was a d.m.e. The counter electrode was a platinum spiral. The saturated calomel electrode (s.c.e.), to which all potentials cited are referred, was a self-contained unit, whose bridge end contained a coarse glass frit and an agar—KCl

plug; its potential was periodically checked.

The d.m.e., which were lengths of Corning marine barometer tubing, had flow rates and drop times in 0.1 M KCl solution at open circuit and a mercury height of 60 cm of (a) 1.155 mg s<sup>-1</sup> and 6.0 s, and (b) 1.95 mg s<sup>-1</sup> and 3.9 s.

The experimental arrangement was periodically checked by running d.m.e. polarograms for 0.5 mM Cd(II) in 0.1 M KCl solution. Here,  $U_{1/2}$  was always  $-0.599 \pm 0.001$  V; the ratio of limiting current ( $I_l$ ) to square root of corrected Hg head ( $h_{corr}^{1/2}$ ) was constant.

### *Test solutions*

The constant ionic strength McIlvaine buffers used were prepared from reagent grade chemicals [14].

The reported analytical data and spectrophotometric assay for the bases and nucleosides used (Calbiochem; Nutritional Biochemicals; P-L Biochemicals) indicated sufficient purity for polarographic study. In order to minimize possible base or nucleoside decomposition in the dissolved state, test solutions were freshly and individually prepared by dissolving weighted amounts of the compounds in the buffer solution. The test solutions were of pH 4.0 or 7.2, 0.1 M ionic strength, and 0.1–1.0 mM in base or nucleoside.

Nitrogen used for deoxygenating was purified and equilibrated by bubbling it successively through acidic V(II) kept over heavily amalgamated zinc, saturated Ca(OH)<sub>2</sub> and distilled water.

### *Procedures*

Test solutions were deoxygenated by N<sub>2</sub> purging and were then examined with N<sub>2</sub> passing over the solution.

*D.c.* polarograms were recorded in the usual manner, employing a natural drop time.

For *a.c.* polarography, a controlled 2 s drop time was used with a *d.c.* potential scan rate of 2 mV s<sup>-1</sup> and a superimposed alternating voltage of 5 mV peak amplitude (3.54 mV rms) and 50 Hz frequency. The phase angle was determined in accordance with the recommended procedure for the PAR apparatus. The phase angle selected for the in-phase current component measurement was that for which the noise, as indicated on the lock-in amplifier galvanometer, was at a minimum; the quadrature current component was recorded at a phase angle 90° from that used to measure the in-phase current component. Quadrature current measurements were made when solution/adsorbate equilibrium had been attained (cf. subsequent discussion on effect of time).

## GUANOSINE—ADENOSINE INTERACTION

### *D.c. polarography*

At pH 4.0, no differences are observable in the *d.c.* polarographic curves for 0.1–0.5 mM adenosine alone and for mixtures of adenosine and guanosine up to a ratio of 1 : 4 adenosine : guanosine, where the adenosine is 0.1–0.5 mM.

In all cases,  $I_1$ , based on the maximum current for the adenosine reduction wave, is diffusion-controlled (linear dependence on  $h_{\text{corr}}^{1/2}$ ). The half-wave potential ( $U_{1/2}$ ) at 25°C is constant at  $-1.32 \pm 0.01$  V, in agreement with a previously observed  $U_{1/2}$  of  $-1.32$  V at 0.1 M ionic strength and 25°C [15]. The diffusion current constant [ $I_d = I_1/c m^{2/3} t^{1/6}$ ] is ca. 13.3 at 0.25 mM adenosine and 12.4 at 0.5 mM; the decrease with increasing concentration is in accordance with previous reports for adenine species.

As expected, no faradaic reduction peak is seen in pH 7.2 solutions of adenosine and guanosine alone or in the mixture, since the guanosine moiety is not reducible within the available potential range in aqueous media and the adenine moiety is reducible only when protonated at N(1) [16].

In solutions containing adenosine, the background decomposition potential is shifted positively owing to the effect of the adenine moiety in lowering the hydrogen reduction overpotential.

#### A.c. polarography

The height of the adenosine in-phase current component faradaic peak seen at pH 4 is greatest at a phase angle of  $-7^\circ$  to  $-15^\circ$ ; the summit potential ( $U_s$ ) of  $-1.37$  V at 25°C is unaffected by the presence of guanosine.

Over most of the available potential range there is a pronounced difference in the quadrature current component between the buffer background solution alone and such solutions containing adenosine or guanosine alone, or mixtures of the two (Figs. 1, 2); the difference is greatest in the potential region centered at  $-0.6$  V, which is near the electrocapillary maximum (e.c.m.) or point of zero charge (p.z.c.). The maximum deviation is observed at a complementary phase angle of  $+75^\circ$  to  $83^\circ$  (cf. the in-phase current component phase angle of

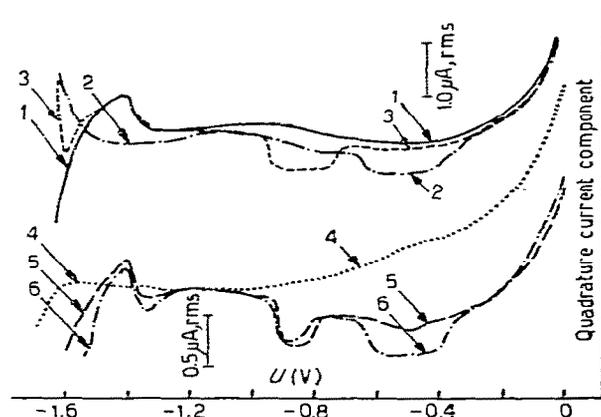


Fig. 1. Alternating current quadrature current polarograms at pH 4.0 and 25°C for: (1) 0.50 mM adenosine; (2) 0.50 mM guanosine; (3) 0.50 mM adenosine plus 0.50 mM guanosine; (4) background buffer solution alone; (5) 0.25 mM adenosine plus 0.50 mM guanosine; (6) 0.25 mM adenosine and 0.75 mM guanosine. Some slight difference between the group of curves 1–3 and that of curves 4 and 5 may be due to a difference in instrument sensitivity. Background curve (4) refers to curves (5) and (6).

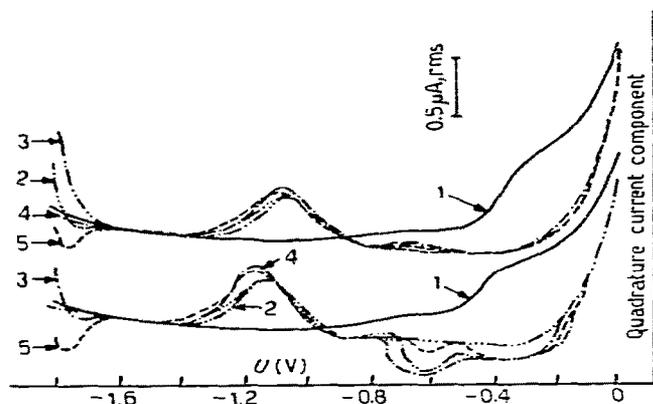


Fig. 2. Alternating current quadrature current polarograms at pH 7.2 for: (1) background buffer solution alone; (2) 0.50 mM guanosine; (3) 0.50 mM guanosine plus 0.25 mM adenosine; (4) 0.75 mM guanosine; (5) 0.75 mM guanosine plus 0.25 mM adenosine. Upper group of curves is at 40°C; lower group is at 25°C.

–7° to –15° mentioned in the previous paragraph), and is about the same in 0.1 mM adenosine and 0.1 mM guanosine, observed separately; the deviation for adenine is less than one-half of that for a similar adenosine concentration. The difference does not increase linearly with guanosine concentration, for example, it is about  $-0.4 \mu\text{A}$  at 0.1 mM and  $-0.8 \mu\text{A}$  at 0.5 mM.

At pH 4.0 and 40°C, adenosine has a  $U_{1/2}$  of  $-1.33 \text{ V}$ , an in-phase  $U_s$  of  $-1.38 \text{ V}$  and a corresponding quadrature  $U_q$  of  $-1.41 \text{ V}$ . Addition of guanosine does not significantly alter these potentials or the corresponding currents. Guanosine alone does not produce current steps at the potentials indicated.

#### Capacitive current pit or well

Adsorption of a compound at a solution|electrode interface generally lowers the magnitude of the differential capacitance of the electrical double layer at the interface and, correspondingly, the intensity of the alternating current which is involved in the charging of the double layer and which, in the absence of a faradaic process, is reflected in the a.c. polarographic quadrature current component. The potential at which the differential capacitance is most reduced, compared to that in the absence of the adsorbate or surface-active species, defines the potential range in which the greatest portion of the latter species is adsorbed. Potentials, at which capacitance or current maxima appear, are those where the most rapid adsorption and/or desorption processes occur; where the current following the peak is below the response for background alone, such current maxima might also indicate a reorientation of the adsorbate. Appearance of a more or less sharply defined well or pit on the current–potential or capacitance–potential curve is generally identified with the occurrence of association between adsorbed molecules to form a surface film [1,17]. For example, in Fig. 3, adsorption is greatest in the potential range around  $U_0$ , where the decrease in the alternating current ( $I_{a.c.}$ ) relative to that for the back-

ground electrolyte solution alone, is greatest; peaks at  $U^+$  and  $U^-$  reflect an increase in  $I_{ac}$  across the interface due to adsorption-desorption with the pit in curve  $c$  resulting from the association of the adsorbed solute molecules. The walls of the pit are at the potentials at which the surface film forms and breaks.

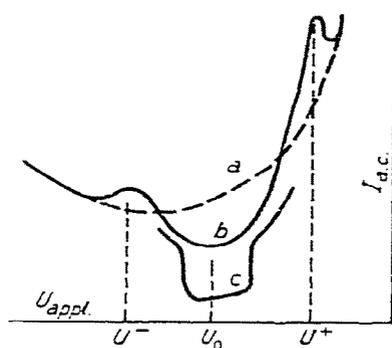


Fig. 3. Dependence of the alternating current,  $I_{ac}$ , on the electrode potential,  $U$ . Alternating current polarograms of: (a) background electrolyte in the absence of specific adsorption; (b) the same solution containing some surface-active substance; (c) the same solution when intermolecular association of adsorbed species occurs on the electrode surface. Taken from Vetterl [1], with some modification.

#### Quadratic current component phenomena

As expected from Vetterl's studies, the nucleosides examined adsorb at the solution/mercury interface in the region of the e.c.m. Pits (cf. previous paragraph) are observed on *a.c.* quadrature current component polarograms of guanosine and guanosine-adenosine mixtures (Fig. 1). Adenosine by itself produces no quadrature pits at concentrations of 1 mM or less at either pH 4.0 or 7.2 at both 25 and 40°C; the general negative displacement from the background curve testifies to the adsorption of the adenosine.

The results seen at pH 4 can be summarized as follows (the items are numbered to facilitate subsequent reference):

(1) At 25°C and guanosine concentration of 0.2 mM or greater, a sharply defined minimum corresponding to a pit is seen in the potential region of the e.c.m. (Fig. 1).

The width of the pit in potential increases with increasing guanosine concentration with eventual diminution in effect (Fig. 4); the midpoint of the pit remains constant at -0.51 V. The depth of the pit in current and its area (current depth  $\times$  potential width) increase with guanosine concentration in the exponential manner characteristic of an adsorption isotherm (Figs. 4 and 5A). The tendency of the area-concentration relation to level off at higher concentrations, presumably due to interfacial saturation with the species being adsorbed, is more evident in the log-log plot of the data (Fig. 5B).

(2) The pit seen with guanosine at 25°C is greatly diminished on addition of adenosine, nearly vanishing at a 2 : 1 molar guanosine-adenosine ratio; at the

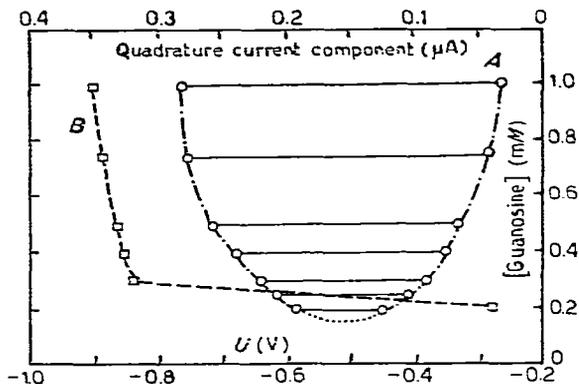


Fig. 4. Variation of the quadrature current pit characteristics with guanosine concentration at pH 4.0 and 25°C: (A) potentials at which the pit begins and ends; (B) mean depth of the pit between its initial and final potentials.

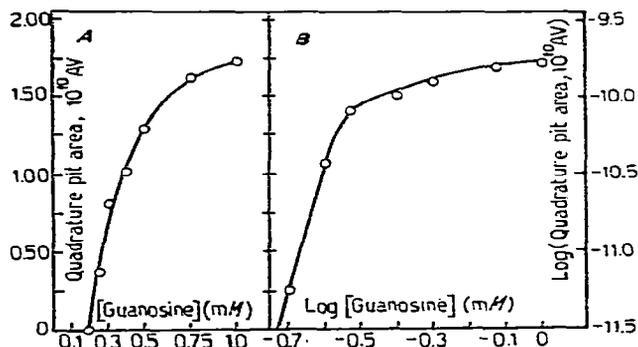


Fig. 5. Variation of the quadrature current pit area (current depth  $\times$  potential width) at ca.  $-0.6$  V with guanosine concentration at pH 4.0 and 25°C: (A) linear plot of data; (B) log-log plot of data.

same time, a pit develops at more negative potential (pit midpoint is at  $-0.83$  V) (Fig. 1). On subsequent addition of guanosine in excess of the 2 : 1 ratio the original pit at  $-0.51$  V redevelops (Fig. 1).

The marked capacitive current excursion seen at ca.  $-1.4$  V for both adenosine alone and adenosine-guanosine mixtures is associated with faradaic reduction of the adenine moiety.

(3) At 40°C, neither of the two pits described in the preceding paragraphs are seen on *a.c.* quadrature current polarograms of guanosine or of guanosine-adenosine mixtures.

At pH 7.2, the following pit phenomena are observed:

(4) The quadrature polarograms for 0.25 mM guanosine and 0.25 mM adenosine alone are nearly the same at 25°C (Fig. 6), indicating almost identical adsorption effects for the two compounds over the potential range.

(5) At higher guanosine concentration at 25°C, e.g., 0.50 mM, the quadrature polarograms have two more or less well-defined pits covering the potential ranges of  $-0.20$  to  $-0.40$  V (Fig. 6). At still higher concentration ( $\geq 0.75$  mM), the two pits merge into a single large pit; since the more negative pit, whose depth increases with increasing guanosine concentration, is the deeper at the concentration of merging, the merged pit shows a two-step depression consisting of a shallow portion centering at  $-0.30$  V and a deeper region centering at  $-0.62$  V with a transition at  $-0.52$  V which is quite close to the e.c.m. potential (Fig. 6). The pit magnitudes increase with increasing guanosine concentration in the exponential shape characteristic of parameters related to adsorption isotherms (Figs. 6, 7).

(6) The guanosine pits at 25°C are diminished on addition of adenosine and disappear as such at a 2 : 1 guanosine : adenosine ratio (Figs. 2, 6). Increase in guanosine above the 2 : 1 ratio causes a reappearance of the second pit (Fig. 2). A pit at more negative potential similar to that centered at  $-0.83$  V, which appeared for guanosine—adenosine mixtures at pH 4 at 25°C, is not seen at pH 7.2 at 25°C.

In the cases of both guanosine alone and guanosine—adenosine mixtures, there is a marked capacitive current excursion starting at about  $-0.9$  to  $-1.0$  V and becoming identical with the background quadrature current component at about  $-1.4$  to  $-1.5$  V (Figs. 2, 6). The peak, whose height increases exponentially with increasing solution concentration of guanosine (Fig. 7), is due to desorption of guanosine from the interface, which also produces an in-phase current component; although adenosine itself produces a peak in this location, the peak heights for guanosine—adenosine mixtures are essentially identical with those for the same concentrations of guanosine alone. As previously noted, adenosine does not produce a faradaic reduction peak at pH 7.2.

(7) At pH 7.2 and 40°C, no quadrature current pits are seen in the  $-0.2$  to  $-0.7$  V range, although adsorption of guanosine and adenosine species in that potential range is evident (Fig. 2). The current peak centered at ca.  $-1.1$  V behaves the same as at 25°C.

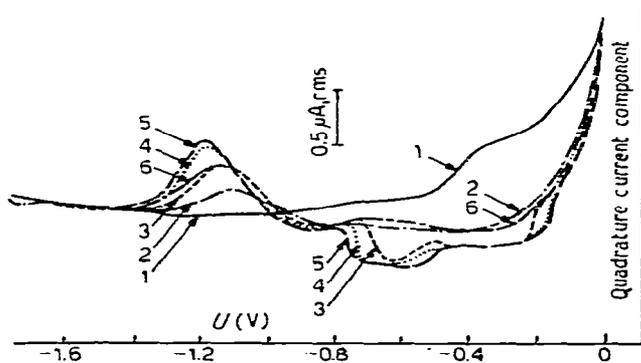


Fig. 6. Alternating current quadrature current polarograms at pH 7.2 and 25°C for: (1) background buffer solution alone; (2) 0.25 mM guanosine; (3) 0.50 mM guanosine; (4) 0.75 mM guanosine; (5) 1.00 mM guanosine; (6) 0.50 mM guanosine and 0.25 mM adenosine.

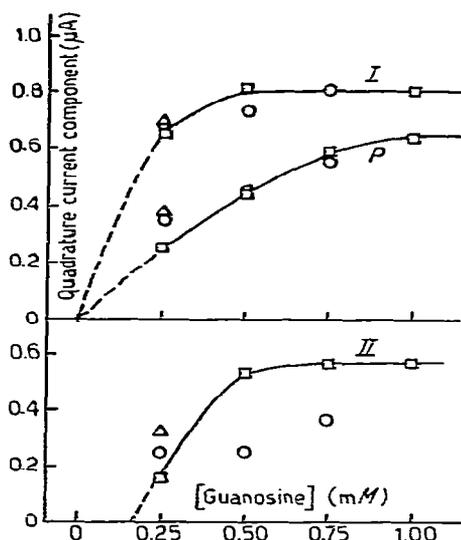


Fig. 7. Variation of the quadrature current and pit depths with guanosine concentration for guanosine alone and in mixture with adenosine at pH 7.2 and 25°C: (I) pit centered at ca.  $-0.3$  V; (II) pit centered at ca.  $-0.6$  V; (P) peak at ca.  $-1.1$  to  $-1.2$  V; ( $\square$ ) guanosine alone; ( $\Delta$ ) 0.25 mM adenosine alone; ( $\circ$ ) guanosine in admixture with 0.25 mM adenosine.

#### *Guanosine self-association*

Result (1) indicates that at pH 4 and 25°C adsorbed guanosine molecules associate. The potential range of the resulting pit being centered at  $-0.51$  V, i.e. in the e.c.m. range, suggests that the association complex has an overall uncharged structure and that the associated guanosine is less polar than guanosine itself. At pH 4.0, guanosine is expected to be unprotonated on the basis of its  $pK_a$  values.

Result (5) indicates formation of an uncharged guanosine association complex at pH 7.2 and 25°C similar to that at pH 4.0.

The guanosine association complex can be formulated as a dimeric species as a result of hydrogen bonding involving the oxygens at C(6) and the hydrogens of the amino groups at C(2), as shown for structure I of Fig. 8. Such intermolecular bonding might stabilize the system against proton addition or loss. While the intermolecular interaction must be strong enough to cause association resulting in a surface film, the relatively low degree of stability of the complex is evident from its failure to be formed at 40°C (results 3 and 7). Analogously, 2 mM adenosine at pH 7 forms a deep pit at room temperature, but the pit disappears at 35°C (Fig. 3 of ref. 2).

#### *Guanosine—adenosine interaction*

The  $pK_a$  values for adenosine indicated that adenosine is partially protonated at pH 4.0 and is uncharged at pH 7.2.

The effects resulting from adenosine addition to a pH 4 solution of guanosine (result 2) are explicable on the basis that the associated adsorbed guanosine species is dissociated due to the formation of a more stable guanosine—adenosine complex, whose stoichiometry is that of two guanosine molecules per adenosine molecule. The adsorption and subsequent association of the latter complex produces a pit at a more negative potential than the pit due to the guanosine complex; more importantly, while the guanosine complex is adsorbed in the e.c.m. potential region, as shown by the pit due to it being centered in that region, the guanosine—adenosine complex is adsorbed at potentials negative to the e.c.m., as expected for a positively charged species.

The failure of the adsorbed guanosine—adenosine complex to produce a pit at more negative potential at pH 7.2 (result 5) as it does at pH 4, could be related to the unprotonated structure present at pH 7.2.

The hydrogen bonding in the complex at pH 7.2 could involve electron-pair donation from the oxygen atoms at C(6) in guanosine and from N(1) and N(7) in adenosine, as shown in structure II in Fig. 8. At pH 4.0, the proton which would normally reside mostly on N(1) of about one-fourth of the adenosine molecules could also reside on N(3) of the adenosine and/or the amino groups at C(2) of the guanosines.

The formation of a guanosine—adenosine complex had been inferred from an apparent capacitive wave which appeared on d.m.e. polarography at ca.  $-1.0$  to  $-1.2$  V [15]; such a wave was also seen in the present studies. As previously noted, desorption of guanosine and guanosine—adenosine complexes produces in-phase and quadrature current components in this potential region.

It should be noted that, as a result of electronic shifts due to the intermolecular hydrogen bonds formed, the basicity ( $pK_a$ ) of the association complexes may differ from those of the individual nucleosides.

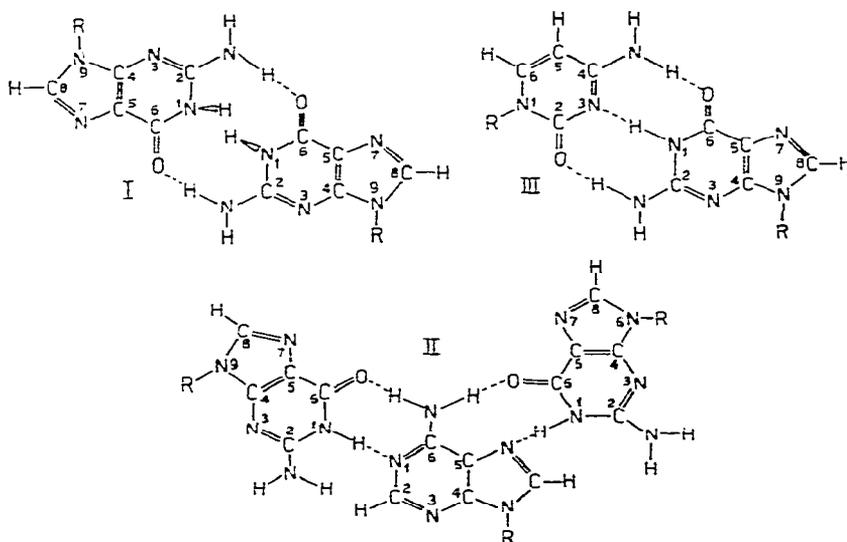


Fig. 8. Possible hydrogen-bonded structures for the association complexes formed by: (I) two guanosine molecules; (II) two guanosine molecules with one adenosine molecule; (III) one guanosine molecule and one cytidine molecule. R represents the ribose moiety.

### Effect of time

Individually deoxygenated buffer and stock guanosine and adenosine solutions were mixed to prepare the test solution. Quadrature current polarograms were then obtained at frequent intervals over a period of up to 10 h. The increase in depth of the pit at ca.  $-0.9$  V (cf. Fig. 1) with time for 2 : 1 and 3 : 1 guanosine—adenosine mixtures is evident from Fig. 9. For the 2 : 1 mixture, an induction period of ca. 2 h was followed by a growth which levelled off after ca. 2 h more. For the 3 : 1 mixture, the induction period was shorter, with a steady state being reached in ca. 3 h. The current peak corresponding to the adenosine faradaic process and the depth of the first pit were constant over the time periods involved.

The data would seem to indicate rapid formation of the associated species involving two guanosine molecules (Fig. 8: species I), followed by the much slower formation of the more bulky species involving two guanosine molecules and one adenosine molecule (Fig. 8: species II), as a result of adenosine reacting with monomeric guanosine in equilibrium with associated guanosine and/or, less likely, directly with the dimeric guanosine.

In any event, irrespective of the mechanistic paths involved, it is advisable to allow the test solution to stand for about 5 h before recording the quadrature polarogram for the equilibrium state. This was done for all solutions for which adsorption and association of complex species were measured.

The change in the double-layer capacitance with time may possibly involve reorientation and/or consolidation, e.g. association, of adsorbed species. Such a process may also account for the slight decrease after 5 h for the 3 : 1 mixture (Fig. 9B).

The fact that guanosine itself reaches interaction equilibrium relatively rapidly may indicate that the guanosine molecules have interacted even in the

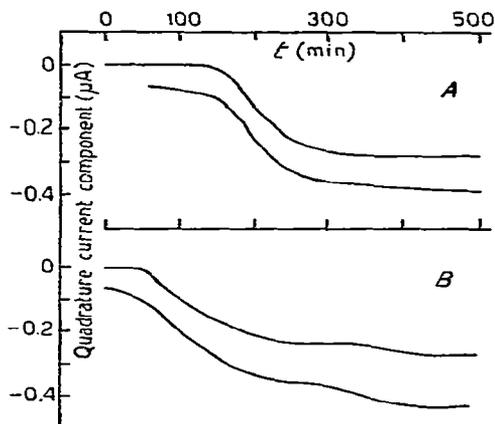


Fig. 9. Variation with time at pH 4.0 and 25°C of the depth of the pit corresponding to adsorption of the guanosine—adenosine complex for: (A) a mixture of 0.25 mM adenosine and 0.50 mM guanosine; (B) a mixture of 0.25 mM adenosine and 0.75 mM guanosine. In each case, the upper curve is for the initial depth of the pit corresponding to adsorption of the complex and the lower curve is for desorption of the complex.

solid state. Unfortunately, experiments which might have shown whether an induction period occurs similar to those of Fig. 9 were not carried out.

## GUANOSINE—CYTIDINE INTERACTION

### *D.c. polarography*

At both pH 4.0 and 7.2 (0.1 *M* ionic strength), the cytidine reduction wave at the d.m.e. is diffusion-controlled (linear variation of  $I_d$  with  $h_{corr}^{1/2}$ ) alone and in mixture with guanosine;  $I_d$  values are those expected for a faradaic  $n$  between 2 and 3;  $U_{1/2}$  is  $-1.31$  to  $-1.35$  V at pH 4.0 and  $25^\circ\text{C}$ ,  $-1.53$  V at pH 7.2 and  $25^\circ\text{C}$  and  $-1.50$  V at pH 7.2 and  $40^\circ\text{C}$ . These results are in conformity with those previously reported for cytidine [16].

### *A.c. polarography*

The in-phase *a.c.* polarograms for cytidine and cytidine—guanosine mixtures show a peak whose height is proportional to cytidine concentration and independent of added guanosine. At both  $25$  and  $40^\circ\text{C}$   $U_p$  is ca.  $-1.40$  V at pH 4.0 and ca.  $-1.60$  V at pH 7.2.

With respect to the quadrature *a.c.* polarograms, cytidine itself shows no pit although it is adsorbed (Fig. 10). Addition at  $25^\circ\text{C}$  of cytidine to a pH 4.0 solution of guanosine at a 1 : 1 molar ratio reduces the potential range covered by the guanosine pit centered at  $-0.51$  V from ca. 370 mV to ca. 200 mV and slightly reduces its depth (Fig. 10). At  $40^\circ\text{C}$  there is no difference between the quadrature patterns for guanosine alone and in mixture with cytidine, except in the potential region beyond  $-1.3$  V where cytidine is reduced.

At pH 7.2 and  $25^\circ\text{C}$ , addition of cytidine to a guanosine solution to a 1 : 1 molar ratio causes the two pits seen in the quadrature curve for guanosine alone to diminish to barely perceptible arcs in the curve. At  $40^\circ\text{C}$  the quadrature curves for guanosine alone and for its mixtures with cytidine are the same except in the potential region beyond  $-1.5$  V where the cytidine is reduced.

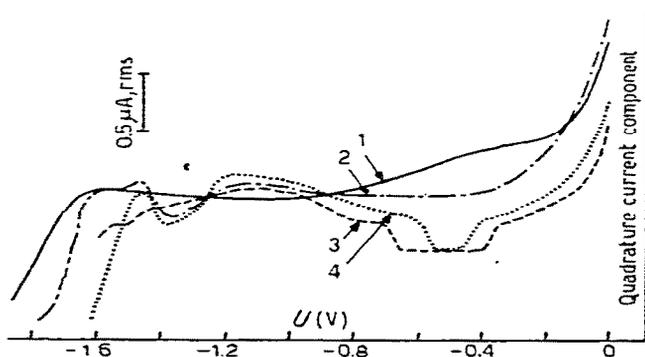


Fig. 10. Alternating current quadrature current polarograms at pH 4.0 and  $25^\circ\text{C}$  for: (1) background buffer solution alone; (2) 0.50 mM cytidine; (3) 0.50 mM guanosine; (4) 0.50 mM cytidine plus 0.50 mM guanosine.

*Guanosine—cytidine interaction*

The data support formation of a 1 : 1 molar association complex between guanosine and cytidine. Based on its  $pK_a$  values, about half of the cytidine would be protonated at pH 4.0, but none would be at pH 7.2. Since the guanosine—cytidine complex seems to be more stable at pH 7.2 than at pH 4.0, based on the relative changes in the guanosine pit patterns at these pH on cytidine addition, the complex formed at pH 7.2 could have a structure (III in Fig. 8) similar to that postulated for the guanosine—cytidine pair on double-helix formation in the nucleic acids in respect to hydrogen-bonding and bridging sites. At pH 4.0, bonding in the complex would be weakened owing to competition for bonding with N(3) in cytidine between the hydrogen on N(1) in guanosine and solution protons whose activity at pH 4.0 is normally sufficient, as noted, to bond to N(3) in about half of the cytidine molecules present.

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