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ELECTROCHEMICAL DETECTION OF ADENOSINE AND OTHER PURINE METABOLITES DURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

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SUMMARY

Electrochemical detection is shown to be an alternative to UV absorbance or fluorescence detection for the analysis of adenosine and other purine nucleosides and bases by high-performance liquid chromatography. In this capacity, the electrochemical detector is generally more sensitive than UV absorbance detectors and, unlike fluorescence detectors is not limited to adenine-containing compounds which must be derivatized with chloroacetaldehyde to form the fluorescent etheno derivative. Minimum detectable amounts were determined to be (pmoles): adenosine, 0.20; deoxyadenosine, 0.50; adenine, 0.05; inosine, 1.0; hypoxanthine, 0.50; guanosine, 0.13. For the adenine-containing compounds, electrochemical detector sensitivity rivals that of the fluorescence detector.

The system described gives excellent separation and quantification of hypoxanthine, adenine, inosine, guanosine, adenosine and deoxyadenosine. Detector response is linear over a wide range of amount injected. The manner in which the electrochemical detector response (chromatographic peak size) varies in response to changes in applied electrode potential and chromatographic buffer pH, electrolyte concentration and methanol content is described. Preliminary data indicate that the system has the potential of functioning in the analysis of biological materials, after applying the proper clean-up procedures to remove interfering material.

INTRODUCTION

Purine and pyrimidine nucleosides and bases are most often detected by UV absorbance when analysis is by high-performance liquid chromatography (HPLC). The fluorescent $1,N^6$-etheno derivatives of adenine-containing compounds allow lower-limits of detectability with a fluorescence detector but derivatization with chloroacetaldehyde is required. Purine bases have been analyzed by Sephadex liquid chromatography with electrochemical detection (ED), but analysis time was extremely long at 7 h per run and the lower-limit of detectability was only 100 pmoles[1]. Although a few purine compounds such as uric acid and several methylxanthines in-

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cluding theophylline and caffeine[2–4] have been quantified using HPLC–ED, the feasibility of using HPLC–ED for purine and pyrimidine nucleosides and bases, in general, has not been explored. In this communication we report a very sensitive HPLC analysis of adenosine, deoxyadenosine, adenine, inosine, hypoxanthine, and guanosine using oxidative electrochemical detection. We also show that the pyrimidines, uridine, cytidine and 2′-deoxyribosylthymine are relatively poorly detected by electrochemical detection (oxidation). A preliminary report of this work has been presented[5].

EXPERIMENTAL

Reagents and chemicals

Water used in this study was doubly distilled, the second distillation being into glass storage bottles at a condensate temperature of 80–85°C. Bottlecap liners were PTFE. All glassware was washed with concentrated nitric acid and well rinsed prior to use. Methanol was HPLC grade from Burdick & Jackson (Muskegon, MI, U.S.A.), nucleosides and bases were from Sigma (St. Louis, MO, U.S.A.), and KH_2PO_4, $K_2HPO_4 \cdot 3H_2O$, and H_3PO_4 were from Mallinckrodt (St. Louis, MO, U.S.A.). Phosphate buffers were adjusted to a particular pH by mixing equimolar solutions of H_3PO_4 and KH_2PO_4 or KH_2PO_4 and K_2HPO_4. The pH of all solutions was determined with a Beckman Model 4500 digital pH meter and Model 39505 combination electrode (Beckman Instruments, Houston, TX, U.S.A.), standardized against commercially available standards. The pH of the 0.050 *M* potassium phosphate buffer is augmented when methanol is added, closely approximating an increase of 0.1, 0.2 and 0.3 pH unit for 5, 10 and 15% methanol, respectively.

A two-stage degassing scheme was used for chromatographic buffers. First, the aqueous phosphate buffer was exhaustively degassed (20 min) under water aspirator vacuum, with rapid magnetic bar mixing. After addition of the appropriate volume of methanol the solution was again mixed and subjected to 1 min of active boiling at room temperature under water aspirator vacuum. This treatment did not result in a significant loss of methanol from the buffer as evidenced by the chromatographic peak retention times remaining constant even though evacuation periods were varied up to 3 min.

HPLC

Analytical column chromatography was accomplished with a system composed of a Milton Roy mini-pump (Lab Data Control, Riviera Beach, FL, U.S.A.), a Rheodyne 7120 sample injection valve (Rheodyne, Cotati, CA, U.S.A.), a Bioanalytical Systems electrochemical detector (LC-4 controller, LC-5A glassy-carbon electrode transducer, and RE-1 Ag/AgCl reference electrode) (Bioanalytical Systems, W. Lafayette, IN, U.S.A.) and an Omniscribe B5117 recorder (Houston Instruments, Austin, TX, U.S.A.) set at a sensitivity of 1 volt full scale. To reduce the system pressure and pump pulsation differential an Altex-Ultrasphere (5 μm, 250 × 4.6 mm I.D.) ODS reversed-phase column (Beckman Instruments) was cut to a 10-cm length. A Kratos S773 UV spectrophotometric detector (Kratos Analytical Inst., Ramsey, NJ, U.S.A.) was used to allow the sensitivities of UV detection and electrochemical detection to be compared.

A pre-saturation column (150 × 4.6 mm I.D.) filled with Whatman Solvecon Silica Gel (37–53 μm) (Whatman, Clifton, NJ, U.S.A.) was placed in the liquid stream immediately before the sample injection valve to saturate the HPLC solvent with silica. In the liquid line between the pre-saturation column and the pump was placed a tee-fitting, which was connected to the bottom of a vertically mounted 15-cm stainless steel pipe (3/8 in. I.D., 1/2 in. O.D.) the top of which was connected to a stainless steel pressure gauge (3000 p.s.i.). This pipe and gauge combination served as a pulsation damping unit.

The sample injection valve, the reversed-phase column and the glassy-carbon electrode were contained within an electrically grounded Faraday cage (35 cm × 35 cm × 65 cm) fabricated from copper sheet. The front of the cage was hinged to allow easy access to the sample injection valve. Most injection volumes were 5–20 μl. Elution was performed in an isocratic mode with potassium phosphate buffers containing from 5 to 15% methanol (see Results) pumped at a rate of 0.50 ml/min. Electrode potentials were positive at the glassy-carbon electrode (oxidation mode) relative to the Ag/AgCl reference electrode.

The exact concentrations of standard solutions (1.02 ± 0.02 m*M*) of the nucleosides and bases were determined spectrophotometrically using millimolar extinction coefficients, ml $\mu mole^{-1}$ cm^{-1}, at spectral maxima, of 15.4 at 259 nm and pH 7 for adenosine[6], 15.9 at 260 nm and pH 7 for deoxyadenosine[7], 12.2 at 248.5 nm and pH 6 for inosine[8], 12.3 at 256 nm and pH 1 for guanosine [6,8], 10.7 at 249.5 nm and pH 6 for hypoxanthine[8], 13.1 at 262 nm and pH 1 for adenine[8,9], 10.0 at 262 nm and pH 2 for uridine[8,10], 13.4 at 280 nm and pH 1 for cytidine[8,10], and 9.65 at 267 nm and pH 7.2 for 2′-deoxyribosylthymine[8,10]. Appropriate dilutions were made for HPLC studies.

RESULTS AND DISCUSSION

A chromatogram showing the separation and ED of hypoxanthine, adenine, inosine, guanosine, adenosine and deoxyadenosine (standard solutions) is presented in Fig. 1. Chromatograms which were in general similar to that of Fig. 1 were ob-

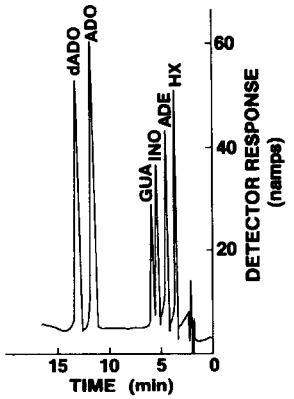

Fig. 1. Chromatogram showing the separation of hypoxanthine (HX, 50 pmoles), adenine (ADE, 50 pmoles), inosine (INO, 1 nmole), guanosine (GUA, 50 pmoles), adenosine (ADO, 200 pmoles) and deoxyadenosine (dADO, 200 pmoles) utilizing electrochemical detection. Electrode potential, 1.5 V; electrochemical detector sensitivity, 100 nA V^{-1}; injection volume, 10 μl; chromatographic buffer, 0.050 *M* potassium phosphate (pH 4.0)–methanol (90:10, v/v).

tained under different conditions of methanol concentration and pH. Chromatogram completion required 45 min with 5% methanol and less than 10 min with 15% methanol. The effect of methanol concentration on the capacity factor, k', is given in Fig. 2 (pH 5.5). For each compound examined, k' decreased with increasing methanol concentration, although the relative elution position of adenine changed rather drastically between 5 and 10% methanol concentration. The relative elution sequence of the compounds was the same at pH 5.5 and 5% methanol (Fig. 2) as at pH 4 and 10% methanol (Fig. 1). The decreased retention time (and capacity factor) of adenine at pH 4 may be related to protonation of the 6-amino group of adenine (pK_a of 4.15[11]).

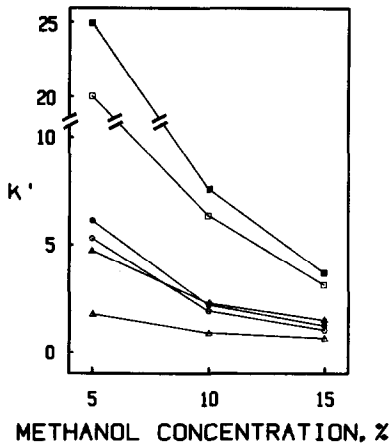

Fig. 2. Effect of methanol concentration (percent by volume) on the capacity factor, k', for hypoxanthine, △; adenine, ▲; inosine, ○; guanosine, ●; adenosine, □; and deoxyadenosine, ■. Chromatographic buffer, 0.050 *M* potassium phosphate (pH 5.5)–methanol (concentration as indicated).

The effect of the magnitude of the electrode potential on the electrochemical detector response (nA sec per pmole injected) was determined for six purine-containing compounds and is shown in Fig. 3. Because it is often necessary to vary the organic component (methanol in this study) of an HPLC buffer in order to achieve a desired chromatographic separation, we also examined the effect of methanol concentration on the detector response (Fig. 3). Three pyrimidine-containing compounds, uridine, cytidine and 2'-deoxyribosylthymine were also studied, but only at a methanol concentration of 10% (Fig. 3B). As shown in Fig. 3, response optima are present. However, detector response occurs over a wide range of electrode potentials for guanosine, hypoxanthine and adenine. Adenosine, deoxyadenosine and inosine yield a detector response only at the higher potentials. We were unable to exceed 1.68 V in this study because of excessive background current (greater than 1 μA). The effect of electrode potential on detector response (Fig. 3) is characteristic of the individual compounds and could be a potential aid in the identification of these compounds.

The study of the effect of methanol concentration on detector response (Fig. 3) shows that while detector responsiveness changed in magnitude for each compound, as the methanol concentration was varied, the shape of each curve and posi-

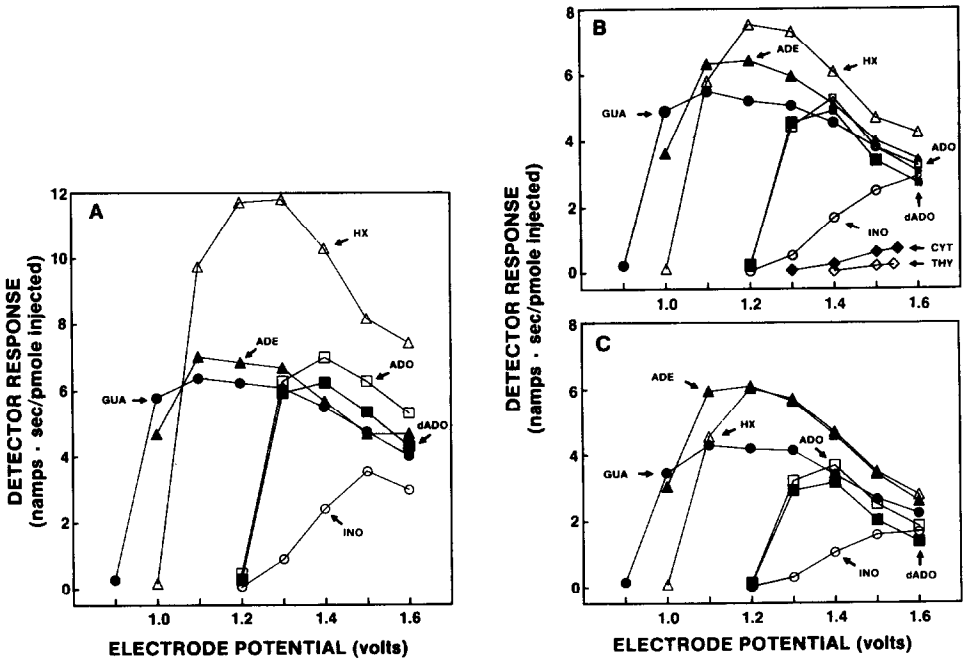

Fig. 3. Effect of the applied electrode potential on the electrochemical detector response (peak area per pmole injected) with (A) 5% methanol, (B) 10% methanol and (C) 15% methanol, for hypoxanthine (HX, 50 pmoles), adenine (ADE, 50 pmoles), guanosine (GUA, 50 pmoles), deoxyadenosine (dADO, 200 pmoles), adenosine (ADO, 100 pmoles) and inosine (INO, 100 pmoles). For cytidine (CYT, 200 pmoles) and 2'-deoxyribosylthymine (THY, 200 pmoles), the effect of electrode potential is shown at 10% methanol only (B). Electrode potential, as indicated on the figure; electrochemical detector sensitivity, 100 nA V^{-1}, except for 2'-deoxyribosylthymine, 20 nA V^{-1}; injection volume, 10 μl; chromatographic buffers, (A) 0.050 *M* potassium phosphate (pH 5.5)–methanol (95:5, v/v), (B) 0.050 *M* potassium phosphate (pH 5.5)–methanol (90:10, v/v), (C) 0.050 *M* potassium phosphate (pH 5.5)–methanol (85:15, v/v).

tions of the maxima changed very little. The most dramatic change (a decrease) in responsiveness which occurred with increasing methanol content was for hypoxanthine. Guanosine, adenosine and deoxyadenosine responsiveness also decreased considerably with increasing methanol concentration, while the electrode responsiveness for adenine remained rather constant. The effect of the methanol content of the chromatographic buffer on the electrochemical detector response at a potential of 1.50 V (data from Fig. 3) is illustrated in Fig. 4.

The effect of methanol on chromatographic peak size is rather complex. Chromatographic peak heights and widths change with the methanol content of the chromatographic buffer. In addition, the "uncompensated resistance" of the electrolyte (chromatographic buffer) between the reference and glassy-carbon electrodes increases with increasing methanol content of the chromatographic buffer, thus decreasing the oxidizing potential actually present at the glassy-carbon electrode. Such a decrease may increase or decrease the detector signal (chromatographic peak size) depending on which compound is being oxidized at the electrode surface and what the applied potential is (see Fig. 3).

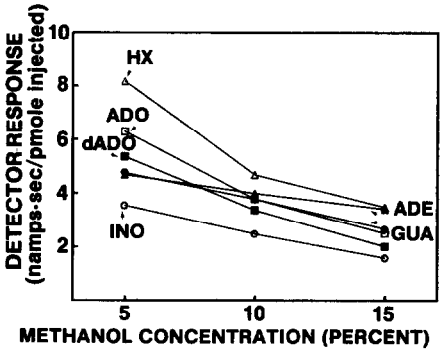

Fig. 4. Effect of methanol content of the chromatographic buffer on the HPLC electrochemical detector response (peak area per pmole injected) for hypoxanthine (HX, 50 pmoles), adenosine (ADO, 100 pmoles), deoxyadenosine (dADO, 200 pmoles), guanosine (GUA, 50 pmoles), adenine (ADE, 50 pmoles) and inosine (INO, 100 pmoles). Electrode potential, 1.50 V; electrochemical detector sensitivity, 100 nA V^{-1}; injection volume, 10 μl; chromatographic buffers, as in Fig. 3.

Uridine, cytidine and 2′-deoxyribosylthymine were also studied with respect to electrochemical detector response as a function of applied electrode potential (Fig. 3B). The response of cytidine was greater than that of 2′-deoxyribosylthymine but both elicited considerably lower response than the purine compounds studied. Uridine was barely detectable.

Detector response was found to be linear over a wide range for each of the six purine compounds studied (data shown only for inosine, Fig. 5). Although electrochemical detector linearity was found to be quite good, caution must be taken when quantifying a compound over an extremely wide range of amount injected because non-linearity which is voltage-dependent can occur, as shown in Fig. 5 for inosine. Although response linearity was observed at an electrode potential of 1.50 V [0.050 *M* potassium phosphate (pH 5.5)–methanol (85:5, v/v)] for amounts of up to 100

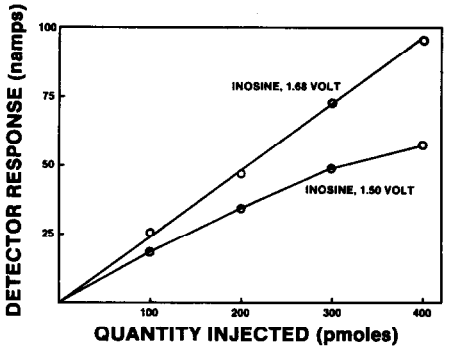

Fig. 5. Effect of electrode potential on linearity of electrochemical detector response (chromatographic peak height) for inosine. Electrode potential as indicated on figure; electrochemical detector sensitivity, 100 nA V^{-1}; injection volumes, 5, 10, 15 and 20 μl; chromatographic buffer, 0.050 *M* potassium phosphate (pH 5.5)–methanol (85:15, v/v).

pmoles of inosine injected (data not shown), a non-linear response occurred when more than 100 pmoles was injected (1.50 V, Fig. 5). However, when the electrode potential was increased into the optimal region for inosine (1.68 V, Fig. 5) the response was linear into the 400-pmole range. It is likely that this phenomenon occurs with other compounds when they are chromatographed over a wide range of amount injected. Response linearity must be examined and if necessary chromatography should be performed with the electrochemical detector set at a potential within the optimal range for the compound of interest. Otherwise, a standard (non-linear) curve must be obtained and used for quantification.

Detector response (nA per pmole injected) for hypoxanthine, inosine and adenosine was found to increase as a function of electrolyte (phosphate) concentration of the chromatographic buffer up to *ca.* 0.04 *M* potassium phosphate and remained constant between 0.04 *M* and 0.07 *M* potassium phosphate (Fig. 6). Because of this finding we extrapolated and employed potassium phosphate at a concentration of 0.050 *M* in the preparation of chromatographic buffers for the study of all of the compounds in this report. This electrolyte effect (Fig. 6) is most probably due to a decrease in the "uncompensated resistance" in the detector flow cell. Retention times of chromatographed compounds were unaffected by phosphate concentration.

The effect of pH of the chromatographic buffer on the electrochemical detector response (nA sec per pmole injected) for hypoxanthine, adenine, adenosine, deoxy-

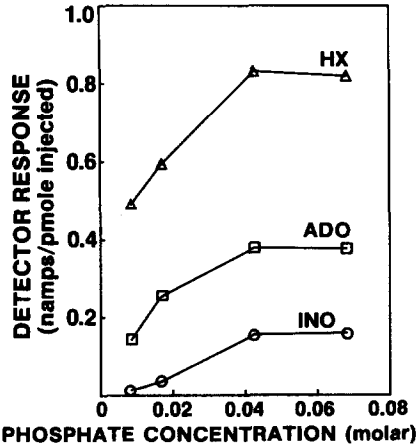

Fig. 6. Effect of chromatographic buffer phosphate concentration on HPLC electrochemical detector response (peak height per pmole injected) for hypoxanthine, adenosine and inosine. Electrode potential, 1.50 V; electrochemical detector sensitivity, 100 nA V^{-1}. Chromatographic buffer composition was potassium phosphate (pH 5.5)–methanol (85:15, v/v); each of the four methanol-containing chromatographic buffers (0.0085 *M*, 0.017 *M*, 0.0425 *M* and 0.068 *M* phosphate, respectively) was prepared by adding 15 volumes of methanol to 85 volumes of pH 5.5 potassium phosphate buffer (0.010 *M*, 0.020 *M*, 0.050 *M* and 0.080 *M*, respectively).

adenosine and inosine is shown in Fig. 7. For hypoxanthine there is a slight decrease in the detector response as the pH is increased from 3.3 to 6.8. For adenine, an identical increase in pH decreases the detector response by one-half. The one-third decrease in detector response with increasing pH for adenosine was very similar to

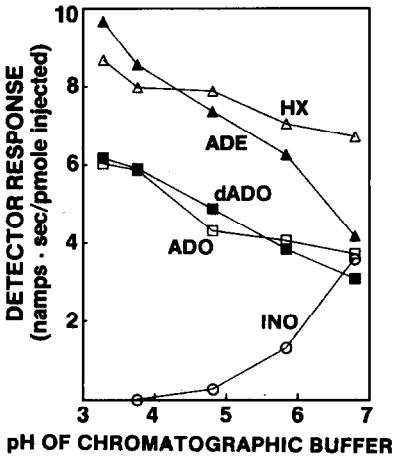

Fig. 7. Effect of chromatographic buffer pH on HPLC electrochemical detector response (peak area per pmole injected) for hypoxanthine (HX, 40 pmoles), adenine (ADE, 50 pmoles), deoxyadenosine (dADO, 200 pmoles), adenosine (ADO, 100 pmoles) and inosine (INO, 200 pmoles). Electrode potential, 1.50 V; electrochemical detector sensitivity, 100 nA V^{-1}; injection volume, 10 μl. Five buffers at different pH values were prepared as usual, 0.050 *M* potassium phosphate (pH 3.0, 3.5, 4.5, 5.5, 6.5, respectively)–methanol (85:15, v/v) resulting in phosphate–methanol chromatographic buffers of 0.0425 *M* phosphate and pH values of 3.28, 3.76, 4.81, 5.84 and 6.80, respectively.

that for deoxyadenosine. In contrast, the detector response for inosine increased many-fold with increasing pH over the pH range 3.8–6.8.

We determined the minimum detectable amount (lower-limit of detection, based on a signal to noise ratio *ca.* 5) of adenosine, deoxyadenosine, adenine, inosine, hypoxanthine and guanosine, comparing the sensitivity of a Bioanalytical Systems electrochemical detector (Table I) and a Kratos S773 UV spectrophotometric detector (Table II). Decreasing amounts of each compound were injected and peak heights were measured, with detectors set at high sensitivity. The relative sensitivity of the two detectors for each of the above purine compounds is given in Table II. For adenine-containing compounds the sensitivity of the electrochemical detector is greater than that of the Kratos S773 UV detector, which is one of the most sensitive UV detectors available at this time. Especially noteworthy is the fact that the minimum detectable amount of adenine is 50-fold lower with ED than with UV spectrophotometric detection (Tables I and II). Furthermore, ED is competitive in sensitivity with fluorometric detection, which requires the reaction of chloroacetaldehyde with adenine-containing compounds to give the fluorescent 1,N^6-etheno derivatives. for example, adenosine and adenine (as the fluorescent 1,N^6-etheno derivatives) are reported to have lower-limits of fluorometric detection of 1 pmole and 5 pmoles, respectively, in one study[12], and 1 pmole for each compound in another study[13]. In comparison, we show that the lower-limits with ED are 0.2 pmole for adenosine and 0.05 pmole for adenine (Table I), without a requisite derivatization step. Guanosine, which does not react with chloroacetaldehyde to form a fluorescent etheno derivative, is detectable electrochemically down to 0.13 pmole, a minimum detectable amount which is 24-fold lower than that attainable by UV spectrophotometric detection

TABLE I

LOWER-LIMIT OF DETECTION USING A UV SPECTROPHOTOMETRIC DETECTOR — COMCAL DETECTOR

All chromatographic conditions were as described in Experimental, except as detailed below. The amount injected, shown below, was judged to be the minimum detectable amount (lower-limit of detection) based on the criterion of a signal-to-noise ratio of *ca.* 5/1. The chart recorder had a full scale dimension of 254 mm.

Compound	*Amount (pmoles)*	*N**	*Sensitivity** (nA V^{-1})*	*Electrode potential (V)*	*Buffer****		*Peak height (mm) (mean ± S.D.)*
					Methanol (%)	*pH*	
Adenosine	0.20	6	1	1.40	10	4.2	17.3 ± 2.2
Deoxyadenosine	0.50	3	2	1.40	10	4.2	17.3 ± 0.6
Adenine	0.050	5	1	1.20	5	4.1	20.4 ± 1.5
Inosine	1.00	4	5	1.50	15	6.8	15.8 ± 1.3
Hypoxanthine	0.50	4	5	1.40	5	4.1	26.5 ± 0.6
Guanosine	0.125	3	1	1.20	5	4.1	20.3 ± 0.6

* *N*, number of replicate injections; all injections were 5 μl, except adenosine, 2 μl.

** Sensitivity; 1 nA V^{-1} is twice the sensitivity of 2 nA V^{-1}, etc.

*** Buffer was prepared by mixing 0.050 *M* potassium phosphate (of pH 4.0 or 6.5) with the appropriate amount of methanol to give the above methanol concentrations (by volume) and pH values.

TABLE II

LOWER-LIMIT OF DETECTION USING A UV SPECTROPHOTOMETRIC DETECTOR—COMPARISON OF ELECTROCHEMICAL AND UV DETECTION SENSITIVITY

Chromatographic conditions were the same as outlined in Table I, except as otherwise indicated in this table. A Kratos S773 UV detector was used. Each compound was analyzed with the UV detector set at the spectral maximum for that compound (as listed in Experimental). The detector was set on the most sensitive setting, 0.001 a.u.f.s. Using the same criterion as in Table I, the amount injected, below, was judged to be the minimum detectable amount (lower-limit of detection). The number of replicate injections was three for each compound except thymidine, four.

Compound	*Amount (pmoles)*	*Volume injected (μl)*	*Peak height (mm) (mean ± S.D.)*	*Relative sensitivity* ED/UV*
Adenosine	2.0	20	18.5 ± 0.3	10
Deoxyadenosine	3.0	15	23.2 ± 1.4	6
Adenine	2.5	25	19.0 ± 1.0	50
Inosine	1.5	15	23.5 ± 0.5	*ca.* 1
Hypoxanthine	2.0	20	22.0 ± 1.0	4
Guanosine	3.0	30	22.5 ± 1.2	24
Thymidine	2.5	5	24.2 ± 1.5	*ca.* 1/8
Cytidine	2.5	5	28.1 ± 1.0	*ca.* 1/4

* Relative sensitivity, ED/UV = $\frac{\text{minimum detectable amount, UV detector}}{\text{minimum detectable amount, electrochemical detector}}$

(Tables I and II). The lower-limits of detection for the pyrimidine nucleosides, 2'-deoxyribosylthymine and cytidine, were not determined with the electrochemical detector. However, the data shown in Fig. 3B indicate that (for the electrochemical detector) the minimum detectable amounts of 2'-deoxyribosylthymine and cytidine were *ca.* 20 and 10 pmoles, respectively, indicating that for pyrimidines the UV detector is more sensitive than the electrochemical detector (Table II).

Of the detection modes available to liquid chromatography, the electrochemical detector is probably the most "temperamental", especially at high electrode potentials such as utilized in the present work (+1.2 to +1.7 V). We did experience problems with both short-term noise and drift when detector sensitivity was set at 10 nA V^{-1} (1 V = full-scale recorder output) or below. The most sensitive detector setting which we found to be practical was 1 nA V^{-1}. Upon applying an electrical potential (+1.2 to +1.7 V) to the glassy-carbon electrode we observed considerable drift in the detector output for 30–120 min, and the higher the applied potential the longer the period of initial drift. Tiny bubbles which could be seen only with a magnifying glass would on occasion collect on the bottom flat surface of the reference electrode and cause short-term noise or drift. Thorough degassing of the chromatographic buffer minimized such problems. Static electricity caused detector noise and made it necessary for us to use a grounded Faraday cage (described in the *HPLC* section) at sensitivity settings of 10 nA V^{-1} to 1 nA V^{-1}. The electrochemical detector was exquisitely sensitive to variations in flow-rate of the chromatographic buffer. Changes of flow-rate with time could cause baseline drift, and flow pulsation associated with each reciprocation of the HPLC pump was a source of short-term noise. Again, the higher the electrode potential and the higher the sensitivity setting of the detector, the greater the noise.

The high oxidation (electrode) potentials used in these studies made it especially necessary to avoid (potentially oxidizable) contaminants. Water, glass-distilled and stored in a polyethylene container, gave a high background current making it impossible to raise the electrode potential above *ca.* 1.2 V. Although we did not resort to distillation of water from alkaline permanganate solution, we did take great care in water preparation and storage, and in the cleaning of all glassware (see Reagents and chemicals).

We observed that electrode responsiveness varied and generally exhibited a slow decay with time. This decay is likely due in part to the adsorption of purines and purine oxidation products to the glassy-carbon electrode[14]. To restore the electrode's ability to oxidize the purine compounds studied, two methods were tried. A mechanical polishing, using a fine alumina obtained from the electrode manufacturer restored electrode responsiveness, but required disassembly of the electrode body. Chemical oxidation, accomplished by flushing the electrode with 1 ml of 1 *M* nitric acid, followed quickly by a second flushing with the chromatographic buffer also restored the responsiveness of the electrode and required less time to accomplish than did mechanical polishing. While electrode responsiveness was quantitatively increased by either of the two treatments, the two methods of electrode restoration do not appear to be equivalent. For example, when we examined the effect of pH on the electrode response to adenosine, we found that the shape of the resulting curve, such as shown in Fig. 7, was dependent on the method by which the electrode had been cleaned. Other investigators have indicated the importance of a thoroughly polished

electrode[15]. The studies presented in this paper were performed with a glassy-carbon electrode cleaned with 1 *M* nitric acid.

The major thrust of this work has been to show that adenosine and other biologically important purine nucleosides and bases are amenable to HPLC analysis with ED. The next logical step is to apply this system to tissue extracts of biological material. Although incomplete, our preliminary work indicates that the system has the potential to function in this capacity. As in all analyses involving trace amounts of metabolites, clean-up procedures to remove the large amounts of nucleotides and other potentially interfering metabolites are necessary[12]. Whether or not the detection limits we report here for pure compounds can be achieved within a biological matrix depends on the extent of sample clean-up achieved, because at such high electrode potentials detector selectivity is limited. However, using procedures for tissue extract preparation and sample clean-up similar to those we described earlier[16], we determined the level of adenosine in rat heart and human plasma using HPLC with electrochemical detection. Our preliminary results are in reasonable agreement with the results of others. For the level of adenosine in rat heart, we found 15, as compared to 24 nmoles per gram of tissue reported by Namm and Leader[17] who used an enzymic method. For the level of adenosine in human plasma, we found 0.02 as compared to 0.07 nmoles ml^{-1} reported by Kuttesch *et al.*[12] who used HPLC with a fluorescence detector. That the compound we measured was indeed adenosine was suggested by (1) its chromatographic retention time and (2) the disappearance of the chromatographic peak after the sample was treated with adenosine deaminase.

Thus, in this report we show that ED of purine nucleosides and bases provides an attractive alternative to UV absorbance or fluorescence detection in HPLC. In this capacity, the electrochemical detector is generally more sensitive than UV absorbance detectors and, unlike fluorescence detectors, is not limited to adenine-containing compounds which must be derivatized with chloroacetaldehyde to form fluorescent etheno derivatives.

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