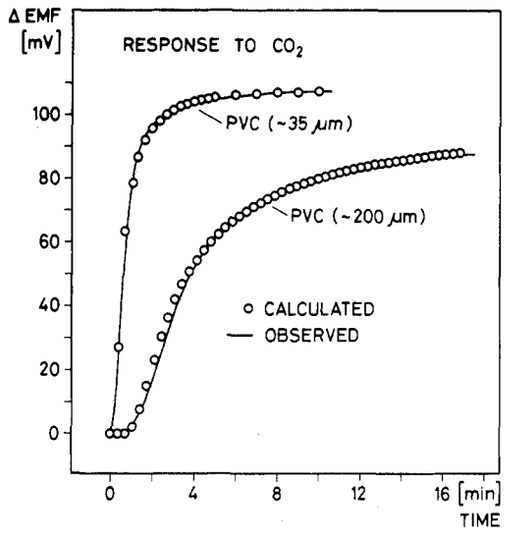

Figure 7. Calculated and observed emf response of CO_2 gas sensors to the primary species. Calculations were based on eq 23 using a time constant ($4d^2/\pi^2 D_{CO_2}$) of 98 s (thin membrane) and 520 s (thick membrane), respectively (*14*).

This result, describing the dynamic response of optimized gas sensors to the primary species, is quite different from an earlier expression derived from a steady-state approach (*4*). However, it nicely conforms to experimentally observed time-response curves (see Figure 7) as well as to a similar theory presented by Buffle and Spoerri (*16*). Since the response to the primary species is usually faster than the equilibration of the sensor with interferents, the approximations involved in the preceding theoretical treatment are absolutely adequate.

From the curve fitting in Figure 7 and from other measurements (*14*) a diffusion coefficient for CO_2 in PVC membranes of $\sim 10^{-7}$ cm^2 s^{-1} is found. This value is considerably lower and the resulting response time is longer than those for the more common membrane materials such as silicone rubber (*4*). The use of PVC membranes in CO_2 sensors is nevertheless attractive because they can easily be modified for simultaneous determinations of pH values and bicarbonate activities (*17*). Due to the exact knowledge of the dynamic response behavior, a further reduction of the required measuring time should become possible by means of computational methods (*18*) or electronic circuits (*19*).

Registry No. CO_2, 124-38-9.

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Caffeine–Picrylsulfonate Liquid Membrane Electrode for Selective Determination of Caffeine in Analgesic Preparations

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A liquid membrane electrode for caffeine prepared from a solution of caffeine–picrylsulfonate ion-pair complex in 1-octanol is developed. It exhibits Nernstian response in the range of 10^{-2}–10^{-6} M caffeine with a cationic slope of 59 mV/concentration decade. The electrode has a wide working pH range (5.5–9.5), fast response time (20 s to 1.5 min), stable response for at least 30 days, and high selectivity for caffeine in the presence of many organic bases. The results obtained for quantitation of 1–1000 μg mL^{-1} of caffeine show an average recovery of 99.5% and a mean standard deviation of 1.3%. Determination of caffeine in some analgesic preparations gives results in good agreement with those obtained by the United States Pharmacopoeia method.

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Caffeine is one of the most important alkaloids consumed in our daily life. It is a mild central nervous system stimulant, is an analeptic, restores mental alertness in fatigued patients, and improves psychomotor coordination. It is present in coffee, tea, cola beverages, and chocolates and used alone or in combination with analgesics in many pharmaceutical preparations for the treatment of headache (*1*). Determination of caffeine in these preparations, however, is commonly beset with many difficulties. The United States Pharmacopoeia (*2*), British Pharmaceutical Codex (*3*), and European Pharmacopoeia (*4*) recommend three methods for the determination of caffeine after prior separation. These are potentiometric titration with perchloric acid in nonaqueous solvents, extraction from strong alkaline media with chloroform, drying, and weighing as free base, and spectrophotometric measurement at 276.5 nm. These methods are time-consuming, nonselective and inapplicable to low levels of caffeine.

A literature survey indicated that caffeine may also be

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determined by more specific titrimetric and spectrophotometric procedures. Reactions of caffeine with tetrazoline blue–tetramethylammonium hydroxide (*5*), malonic acid–acetic anhydride (*6*), strong alkalies–diazotized sulfanilic acid (*7*), and acetyl acetonate–*p*-(dimethylamino)benzaldehyde reagents (*8*) develop measurable colors with maximum absorption at 450–600 nm. Spectrophotometric methods based on redox reactions with either cerium(IV) (*9*) or hypochlorite (*10*) have also been reported. Most of these methods involve reactions under drastic conditions using strong alkalies, strong acids, high temperatures, and long reaction times which make the accuracy and precision of the results highly influenced by variation in the reaction conditions.

Determination of caffeine by methods involving reactions conducted under mild conditions have been advocated. Hexabromotellurate (*11*) and molybdophosphate (*12*) give water-insoluble precipitates with caffeine whose solutions in organic solvents can be spectrophotometrically measured. Precipitation of caffeine with either IBr–HBr (*13*) or lead picrate (*14*) isolation of the precipitates, followed by iodometric or complexometric titration of the excess reagent in the filtrate, respectively, has been suggested. These methods involve several manipulation steps, applicable to relatively high concentrations of caffeine, and entail isolation of the precipitates in pure form. Proton magnetic resonance (*15*) and high-pressure liquid chromatography coupled with spectrophotometry (*16, 17*) have been used for caffeine quantification without pretreatment steps.

On the other hand, ion-selective electrodes with liquid and poly(vinyl chloride) membranes have been developed for direct potentiometric determination of some alkaloids (*18–21*). These monitoring systems have many advantages in terms of simplicity, selectivity, applicability to samples of different nature, and possible interfacing with automated systems. However, no membrane electrode is, so far, available for the determination of caffeine. The present work thus describes the first electrode introduced for caffeine determination based on the use of caffeine–picrylsulfonate ion pair complex in 1-octanol as a liquid membrane. The electrode exhibits high sensitivity, reasonable selectivity, and long-term stability. It has satisfactorily been used for the determination of caffeine in some pharmaceutical preparations. The obtained results match with those obtained by the official methods.

EXPERIMENTAL SECTION

Apparatus. Potentiometric measurements were made at a constant temperature in the range of 25–30 °C with an Orion microprocessor ionalyzer (Model 901). Caffeine–picrylsulfonate liquid membrane electrode in conjunction with an Orion double junction Ag–AgCl reference electrode (Model 90-02) with 10% KNO_3 in the outer compartment was employed. An Orion combined glass calomel electrode (Model 91-00) was used for pH adjustment. The infrared (IR), mass (MS), and nuclear magnetic resonance (NMR) spectra were recorded with a Unicam 200G IR spectrometer, a Varian CH-7 mass spectrometer, and a Varian EM-390 90-MHz NMR spectrometer, respectively.

Reagents. All solutions were prepared with deionized twice-distilled water and analytical reagent grade substances, unless otherwise stated. The organic solvents were twice-distilled reagent grade. Caffeine and picrylsulfonic acid were obtained from Sigma Chemical Co. (St. Louis, MO). A 10^{-2} M caffeine stock solution was prepared by dissolving 1.942 g of pure anhydrous caffeine base in 500 mL of 10^{-1} M hydrochloric acid solution, the pH was adjusted to 1.5, and the solution was diluted to exactly 1 L with twice-distilled water. Dilute solutions (10^{-3}–10^{-6} M) were prepared by appropriate dilution and kept in airtight containers. Pharmaceutical preparations containing caffeine were obtained from local drug stores.

Caffeine–Picrylsulfonate Ion Pair. A 30-mL aliquot of 10^{-1} M aqueous caffeine hydrochloride solution and 50-mL aliquot of 10^{-1} M aqueous picrylsulfonic acid solution were mixed and stirred for 20 min. The yellow precipitate of caffeine–picrylsulfonate complex was filtered with a G-3 sintered glass crucible, washed with twice-distilled water followed by ethanol, dried at 100 °C for 1 h, and ground to fine powder.

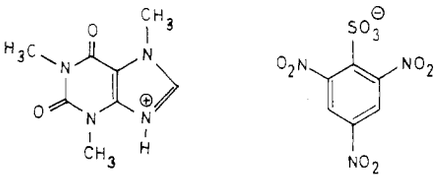

Figure 1. Caffeine–picrylsulfonate ion-pair complex.

Caffeine–Picrylsulfonate Liquid Membrane Electrode. An Orion liquid membrane electrode barrel (Model 92) was used as the electrode assembly with an Orion 92-05-04 porous membrane. The internal reference solution was a mixture of equal volumes of 10^{-2} M caffeine hydrochloride and potassium chloride. The liquid ion exchanger was 10^{-2} M caffeine–picrylsulfonate ion pair in 1-octanol. The electrode was conditioned by soaking in 10^{-2} M caffeine hydrochloride solution for 3 days before use and was also stored in the same solution when not in use. The operative lifetime of the electrode was 4 weeks with daily usage of at least 6 h.

Electrode Calibration. Fifteen-milliliter aliquots of 10^{-2} to 10^{-6} M caffeine hydrochloride solutions were transferred to 100-mL beakers; the pH was adjusted to 6–8.5 by addition of sodium hydroxide solution. The caffeine–picrylsulfonate liquid membrane electrode was immersed in conjunction with a double junction reference electrode (Orion 90-02) into the solutions. The potentials of the stirred solutions were recorded when becoming stable and were plotted as a function of logarithm of caffeine concentration. The graph was used for subsequent determination of caffeine-containing samples.

Determination of Caffeine in Drugs. The contents of five tablets or two ampules were homogenized and a weighed quantity equivalent to one tablet or one suppository was treated with a 50-mL aliquot of 10^{-2} M hydrochloric acid. After the sample was gently heated to about 60 °C for 10 min and cooled, the solid paraffin wax in the suppository was skimmed from the top of the sample solution. The solution was then transferred to a 100-mL volumetric flask and the flask filled to the mark with twice-distilled water. A 15-mL aliquot of the solution was transferred to a 100-mL beaker and the pH adjusted to 6–8.5 with sodium hydroxide. The caffeine–picrylsulfonate liquid membrane electrode was immersed in conjunction with a double junction reference electrode into the solution, the potential was measured after stable reading and compared with the calibration graph.

RESULTS AND DISCUSSION

Nature and Composition of the Membrane. Caffeine readily reacts with picrylsulfonic acid to form a stable 1:1 ion pair complex. The elemental analysis data and the signals appear in the mass spectrum (MS) of the reaction product at m/z corresponding to $(M - 1)^+$, M^+, and $(M + 1)^+$ agree with the composition $C_{14}H_{13}N_7O_{11}S$. The most significant and prominent absorption bands appear in the infrared spectrum (IR) of this compound, but not displayed in the spectra of the reactants are those at 2720 cm^{-1} and 1150 cm^{-1} due to stretching vibrations of protonated imino group (=NH^+) and sulfonate group ($-SO_3^-$), respectively. The nuclear magnetic resonance spectra (NMR) of pure caffeine and its picrylsulfonate ion pair complex show that the four singlet signals displayed at δ 3.2, 3.4, 3.9, and 8.0 ppm, due to three N–CH_3 and olefinic hydrogen atoms of caffeine, appear without splitting at almost the same position in the spectrum of the complex beside two additional singlets at δ 8.9 and 3.7 ppm due to aromatic and NH hydrogen atoms, respectively. This indicates that the unalkylated nitrogen in the caffeine molecule is the proton acceptor center in the ion-pairing reaction. The structure of the complex may thus be represented as shown in Figure 1.

Caffeine–picrylsulfonate ion-pair complex is sparingly soluble in water but dissolves easily in some water-immiscible

Table I. Response Characteristics of Caffeine–Picrylsulfonate Liquid Membrane Electrode at 25 °C in Some Organic Solvents

parameter	nitrobenzene	benzyl alcohol	1-octanol
Slope, mV/log C	35.5	41.7	59.0
std. dev, mV	1.5	1.3	0.7
corr coeff, r	0.998	0.997	1.000
intercept, mV	62.1	60.2	70.1
lower limit of linear range, M	5×10^{-5}	3×10^{-5}	2×10^{-6}
detection limit, M	10^{-5}	10^{-5}	10^{-6}

organic solvents such as 1-octanol, nitrobenzene, and benzyl alcohol. Solutions of the complex in these solvents are examined as liquid membranes for potentiometric determination of caffeine using the electrochemical cell represented by eq 1. The emf is measured as a function of the logarithm of

Ag/AgCl 10^{-2} M KCl 10^{-2} M caffeine	10^{-2} M caffeine–picrylsulfonate in organic solvent	caffeine in test solution	SCE (1)

caffeine concentration according to eq 2, where E_0 is the

$$E = E_0 + 0.059 \log [a_{\text{caffeine}} + K_{ij}(a_j)^z] \quad (2)$$

conditional standard potential of the electrode under the conditions used in the cell, K_{ij} is the selectivity coefficient, a_{caffeine} and a_j are the activities or concentrations of protonated caffeine species and the foreign interfering substance having a charge z and present in the test solution, respectively.

Response Characteristics of the Membrane. The response characteristics of electrodes incorporating 10^{-2} M caffeine–picrylsulfonate complex as an electroactive material in 1-octanol, benzyl alcohol, and nitrobenzene solvents are evaluated at 25 ± 0.5 °C. The least-squares analysis of the data is given in Table I. These data demonstrate the suitability and sensitivity of these membranes for the determination of caffeine and the significant role of the solvent on both the slope of the calibration graph and the limit of detection. The lower limit of usable range for each electrode shown in Table I is based on the detection limit recommended by IUPAC (*22*). The reproducibility cited represents data collected over a period of 6 months from 15 different electrodes. It can be seen that 1-octanol gives a fairly stable and sensitive membrane with Nernstian response for caffeine in pure aqueous solutions over 4 orders of magnitude of concentration (Figure 2). No change in the response behavior of the electrode is noticed either by measuring caffeine in 0.1 M potassium nitrate background or changing the concentration of the complex in 1-octanol over the range of 10^{-2} to 10^{-3} M. This electrode is used for subsequent investigation.

Response Time and Stability of the Membrane. The time required for the caffeine–picrylsulfonate membrane electrode to reach a value of ±1 mV from the final equilibrium potential after successive immersion in different caffeine solutions each having a 10-fold difference in concentration is measured. The time required to achieve ±1 mV of the steady potential by a rapid 10-fold increase of caffeine concentration to the same solution is also monitored. Both results indicate an average response time of 20 s for solutions $>10^{-3}$ M and 1.5 min for solutions $<10^{-4}$ M (Figure 3). Electrode aging has no effect on the response time. On the other hand the electrode exhibits a day-to-day reproducibility within ±2 mV for caffeine concentrations in the range of 10^{-2}–10^{-5} M. The potential reading increases ~10 mV after 1 month. The average slopes of the calibration graph in the first, second, and third days after preparation are 55.7, 57.7, and 59 mV/concentration decade, respectively. The slope remains

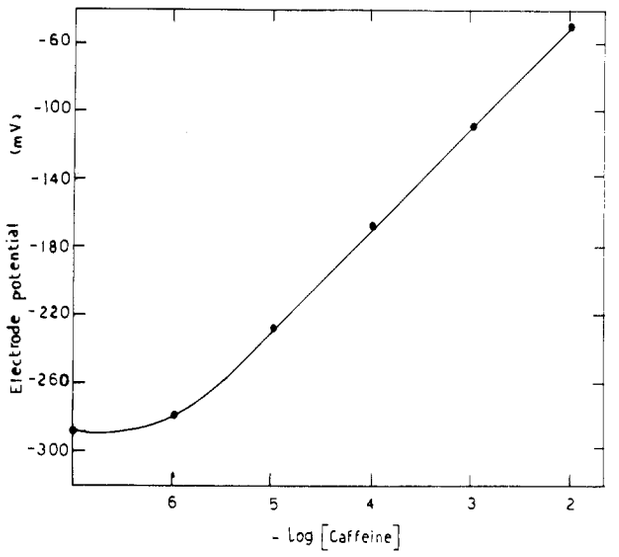

Figure 2. Calibration curve for caffeine at pH 6–8 using caffeine–picrylsulfonate liquid membrane electrode.

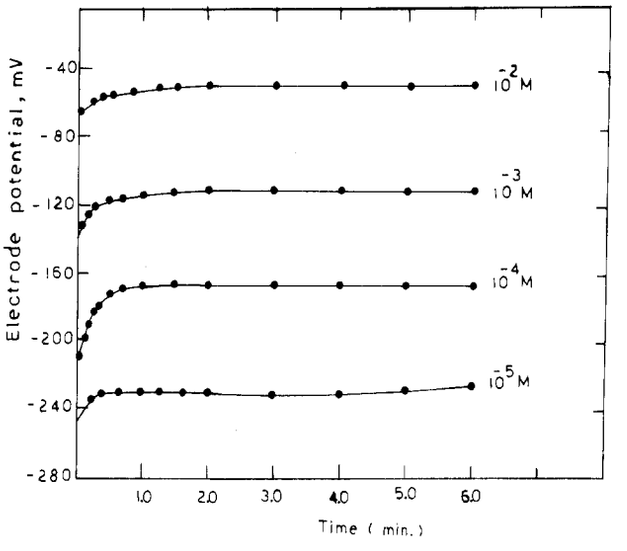

Figure 3. Response time of caffeine–picrylsulfonate liquid membrane electrode for different caffeine concentrations at pH 6–8.

constant after the third day for at least 3 weeks and then declined to 57 mV/concentration decade. After 4 and 6 weeks, the slopes become 56.7 and 53.2 mV/concentration decade, respectively. The electrode can be used for at least 1 month before renewal of the liquid membrane.

Effect of pH and Diverse Compounds. The effect of pH on the response of the electrode for different caffeine concentrations is shown in Figure 4. The pH of the initial caffeine solutions is altered by addition of sodium hydroxide and hydrochloric acid solutions. No change of the electrode potential is observed over the pH range of 5.5–9.5. The potential difference does not exceed 2 mV within the entire range of pH over the concentration range of 10^{-2}–10^{-6} M. During the operative life of the electrode, no significant change in the potential–pH behavior is noticed.

The response of the electrode for caffeine is also examined in the presence of some cationic and anionic organic compounds. The potential displays by solutions each containing 10^{-2} M of the foreign compound with variable caffeine concentrations in the range of 10^{-2}–10^{-5} M is measured. The selectivity coefficients are calculated by using the method of Srinivasan and Rechnitz (*23*)

$$K_{ij} = a_i/a_j^{1/z\pm} [10^{\Delta E/S} - 1] \quad (3)$$

Table II. Selectivity Coefficient for Caffeine–Picrylsulfonate Liquid Membrane in 1-Octanol

interfering compound (j)	selectivity coefficient (K_{ij})
glycine	7.2×10^{-2}
diethylamine	1.2×10^{-1}
triethanolamine	2.0×10^{-1}
urea	1.3×10^{-2}
piperidine	3.2×10^{-3}
ammonium acetate	2.7×10^{-3}
sodium chloride	2.8×10^{-3}
potassium chloride	4.4×10^{-3}
barium chloride	2.8×10^{-1}
nicotinic acid	4.1×10^{-1}
nicotine	3.4×10^{-1}
ephedrine	1.5
quinine	1.3
bruccine	1.6
strychnine	3.5

Table III. Microdetermination of Caffeine Using Caffeine–Picrylsulfonate Liquid Membrane Electrode

	calibration graph method		known addition method	
weight added, $\mu g\ mL^{-1}$	recovery,[a] %	std dev, %	recovery,[a] %	std dev, %
0.65	98.4	2.4	97.3	2.3
1.03	98.3	1.4	98.4	1.1
2.59	99.8	1.6	98.8	1.5
6.47	100.4	1.6	99.3	1.4
10.33	97.3	1.4	98.5	1.8
25.33	98.2	1.5	99.1	0.9
38.67	101.0	1.2	100.1	1.1
64.67	99.3	1.4	99.5	1.5
103.33	100.5	1.7	100.5	0.9
168.00	99.8	1.7	100.3	1.1
258.67	98.0	1.2	99.5	1.6
388.00	101.5	0.9	101.1	1.5
491.33	102.0	0.8	101.6	1.1
647.33	99.8	1.1	99.8	0.9
1035.33	99.9	0.9	98.9	0.9

[a] Average of three measurements.

where ΔE is the change in potential in the presence of the foreign compound $j^{z\pm}$, S is the slope of the calibration curve for caffeine, and a_i and a_j are the concentrations of caffeine and the foreign compounds, respectively. The results obtained (Table II) show that no significant effect is caused by many organic bases and inorganic salts. Some alkaloids interfere only when present at concentration levels at least 10 times greater than those for caffeine. Theobromine and theophylline

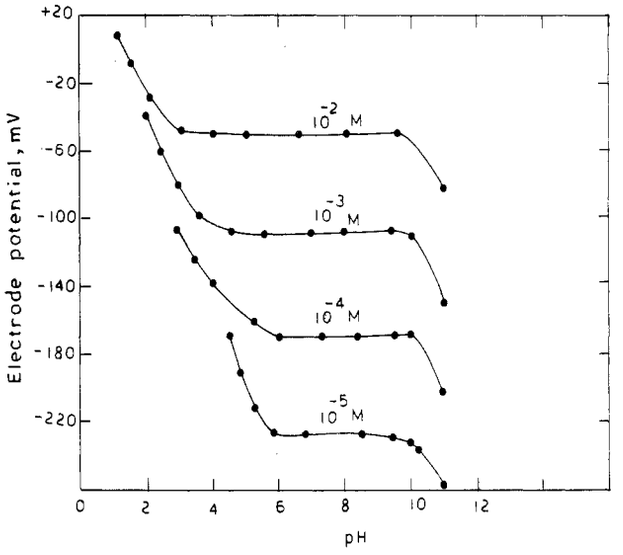

Figure 4. Effect of pH on the potential of caffeine–picrylsulfonate liquid membrane electrode.

seriously interfere ($K_{ij} > 7$) and should be removed before caffeine quantification.

Determination of Caffeine. Caffeine solutions at the concentration range of 0.6–1000 $\mu g\ mL^{-1}$ are prepared from pharmaceutical grade and determined by direct potentiometry using a caffeine–picrylsulfonate liquid membrane electrode. The potentials displayed by these solutions are compared with a calibration graph to asses the accuracy and reproducibility. The results obtained (Table III) for 15 samples, each in triplicate, show an average recovery of 99.6% and a mean standard deviation of 1.4%. Similar results (average recovery 99.5%, mean standard deviation 1.2%) are obtained when using the known addition (spiking) technique. A number of pharmaceutical diluents, excepients and analgesics commonly used in drug formulations, have also been examined for their effect on the electrode response. No interference is caused by aspirin, phenacetin, accacia, sucrose, tween-80, carboxymethylcellulose, lactose, cocoa butter, ethylene glycol, and paraffin oil at levels far in excess of those normally found in drugs (~200 mg).

Caffeine in some pharmaceutical analgesic preparations is determined. It is known that ergotamine tartrate in combination with caffeine is commonly used in some pharmaceutical preparations to abort vascular headaches such as migraine and cluster headaches (histamine cephalalgia). Some of these preparations have similarly been assayed by the present procedure. Tablets and suppositories are treated with hy-

Table IV. Determination of Caffeine in Some Analgesic Preparations Using Caffeine–Picrylsulfonate Liquid Membrane Electrode

			electrode method		USP method (2)	
preparation	source	labeled active ingredients	caffeine recovery,[a] %	std dev, %	caffeine recovery,[a] %	std dev, %
Optalidon (tablet)	Sandoz, Ltd., Switzerland	caffeine 25 mg, propyphenazone 125 mg, butalbital 25 mg/tablet	101.1	1.8	100.9	2.3
Excedrin (tablet)	Bristol-Myers Co., New York	caffeine 65 mg, acetaminophen 250 mg, aspirin 250 mg/tablet	98.7	1.2	101.5	2.0
Migrainil (tablet)	The Nile Pharm & Chem. Co., Egypt	caffeine 50 mg, ergotamine tartrate 1 mg, meprobamate 150 mg, analgin 200 mg, pentobarbital sodium 10 mg/tablet	100.2	1.9	99.8	2.3
Cofergot (suppository)	Sandoz, Ltd., Switzerland	caffeine 100 mg, ergotamine tartrate 2 mg, butalbital 100 mg/suppository	100.9	2.6	99.7	3.0

[a] Average of three measurements.

drochloric acid, heated at 60 °C, and diluted with double distilled water and the potential of their solutions is measured after pH adjustment to 7-8. The results obtained show an average recovery of 100.2% and a mean standard deviation of 1.8% (Table IV). The U.S. Pharmacopoeia method (2) which involves prior extraction followed by potentiometric titration with perchloric acid in benzene-acetic anhydride solvent is also used for comparison. A good agreement between the results obtained by both methods is obtained (Table IV). The present method, however, offers several advantages in terms of simplicity, selectivity, and precision.

Registry No. Caffeine, 58-08-2; picrylsulfonic acid, 2508-19-2; caffeine-picrylsulfonate, 94944-47-5.

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Separation and Determination of Polythionates by Ion-Pair Chromatography

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Reversed-phase ion-pair chromatography with conductivity detection provides a rapid and efficient technique for the quantitative separation of polythionates, sulfate, thiosulfate, and dithionate. Tetraalkylammonium salts were used as ion-pair reagents. Separations were accomplished in less than 15 min, with detection limits in the low parts-per-million range, except for $S_5O_6^{2-}$. Retention was dependent on the nature of both the cation and the anion of the ion-pair reagent.

Reversed-phase ion-pair chromatography has proven to be a valuable tool for the separation of organic solutes (*1*, *2*). However, little has been undertaken utilizing this technique for inorganic anions. Reeve attributes this to the poor detectability of most inorganic ions by the 254-nm ultraviolet detector prevalent in high-performance liquid chromatography (HPLC) systems (*3*). In a recent improvement, UV visualization of inorganic anions has been accomplished by using UV-absorbing quaternary ammonium ion-pair reagents (*4*). A convenient alternative is offered by ion chromatography utilizing conductivity detection (*5*) which offers specificity for ionized species. Further specificity is achieved with amperometric detection, which is sensitive to species that are electroactive (*6*). These detection methods are also proving to be useful for ion-pair chromatography of inorganic ions, especially those that are strongly retained by conventional ion exchange resins. This technique has been utilized for the trace determination of various common inorganic ions in aqueous solution (*7*, *8*).

The separation and determination of polythionates, especially in the presence of thiosulfate, has long been a difficult analytical problem. The motivation behind this work was to develop an analytical technique to quantitatively determine the stoichiometry of the oxidation of thiosulfate by a chelated osmium cation, $[Os(bpy)_3]^{3+}$ (bpy, 2,2'-bipyridyl). Presumably, the major products would be tetrathionate and $[Os(bpy)_3]^{2+}$. This was an adjunct experiment to a study of the kinetics and mechanism of this system. Since the stoichiometry is necessarily studied under conditions where thiosulfate is in excess, and also it was unclear how the osmium might interfere, the classical methods of analysis by sulfitolysis (*9*) and cyanolysis (*10*) were not feasible.

A vast improvement on these methods is the usage of HPLC to separate these species. Chapman and Beard (*11*) used an activated carbon column with 254-nm UV detection; their system was quite insensitive to trithionate, however. Wolkoff and Larose (*12*) suggested a method utilizing fluorescence detection; however, their technique requires postcolumn reaction for the thionates to be detectable. Reeve (*3*) utilized ion-pair chromatography with low wavelength UV detection; he was unable to detect nonchromophores such as sulfate and dithionate which may be present in these reaction mixtures. In a recent paper Takano et al. (*13*) used a polarographic technique to analyze the fractions coming off an anion exchange column, yet they were unable to separate tri- and

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