prepared with I had faster dissolution than the tablets with anhydrous lactose. The dissolution rate of phenobarbital tablets with anhydrous lactose was higher than that of tablets with I. This result may be due to the greater hardness of tablets with I. This hardness was needed to produce satisfactory tablets. The dissolution rate of benzoic acid tablets with I was considerably less than that of tablets made with anhydrous lactose. This finding can be attributed to a further decrease in dissolution medium pH due to the hydrolysis of I to gluconic acid. The results of the dissolution studies are shown in Figs. 1–4.

Aspirin, ferrous sulfate, and phenobarbital tablets prepared with I, anhydrous lactose, and spray-dried lactose and plain tablets of I were stored at 95% relative humidity for 2 months. Mold growth was observed on aspirin tablets and phenobarbital tablets prepared with anhydrous lactose and spray-dried lactose, respectively. None of the tablets prepared with I showed any signs of mold growth.

A comparative study was conducted at various humidity and temperature conditions on the stability of aspirin in a mixture with I and other excipients such as anhydrous lactose, spray-dried lactose, mannitol, and sorbitol. Aspirin hydrolyzed less when mixed with I as compared to other excipients (Figs. 5–9). Mannitol, which is an excellent excipient for moisture-sensitive drugs due to its water-repellent properties (21), contributed more to the hydrolysis of aspirin as compared to I.

No attempt was made to study aspirin–sorbitol mixtures at higher temperatures because a preliminary investigation showed excessive hydrolysis of aspirin in such mixtures.

These results on the stability of aspirin indicate that I probably takes up the environmental moisture for its own hydrolysis into gluconic acid, thereby preventing the hydrolysis of aspirin. A similar mechanism is expected for the stability of other drugs that deteriorate in moisture. A detailed study on this aspect is being conducted.

A preliminary *in vivo* bioavailability study was conducted to obtain information on the effect of I on drug absorption. The results shown in Fig. 10 do not indicate any inhibitory effect of I on aspirin absorption. Detailed bioavailability studies are being conducted on various drugs in combination with I using different test animals. Compound I and anhydrous lactose seem to prolong blood aspirin levels for a greater period as compared to pure aspirin and aspirin–starch mixtures.

Compound I merits serious consideration for use as a direct compression excipient. It can effect compression of problem drugs at relatively low concentrations and yields tablets possessing desired characteristics for pharmaceutical use. The enhancement of stability of moisture-sensitive drugs with I is an additional advantage.

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Electrochemical Analysis of the Cephalosporin Cefamandole Nafate

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Abstract □ The polarographic assays for cefamandole sodium and its formyl ester, cefamandole nafate, are described. Controlled potential coulometry is used as an absolute method for the assignment of purity of these compounds without the need for a reference material. The precision, accuracy, and selectivity of these assays were better than for the microbiological autoturbidimetric and automated iodometric assays. NMR, TLC, GC, and polarography are used to detect and quantitate likely impurities and degradation products.

Keyphrases □ Cefamandole nafate—polarographic analysis, prepared samples □ Polarography—analysis, cefamandole nafate, prepared samples □ Antibacterials—cefamandole nafate, polarographic analysis, prepared samples

Cefamandole (I) has *in vitro* activity against various Gram-positive and Gram-negative bacteria (1–3). A formyl ester, cefamandole nafate (II), was prepared for use in the clinical formulation because it can be purified in a crystalline form having good long-term stability. The ester is hydrolyzed rapidly to cefamandole *in vivo* (4) or in basic aqueous solution (5). Title 21 of the Code of Federal Regulations, Part 442, Cepha Antibiotics, generally provides for both microbiological and chemical assay procedures for cephalosporin antibiotics. However, the microbiological assay results are considered conclusive even though this assay is not specific for any one compound. This paper focuses on the additional tests necessary to characterize completely antibiotic materials such as cefamandole and cefamandole nafate.

Electrochemical techniques are uniquely suited for the assay of compounds containing reducible or oxidizable functions. These include the cephalosporin antibiotics

having a thioether linkage to a substituent at the 3-cephem position. Specifically, cefamandole and cefamandole nafate are easily reduced at the dropping mercury electrode.

This paper reports the polarographic reduction characteristics of these compounds and describes the use of polarography in analytical control and stability procedures. Controlled potential coulometry is proposed as an absolute method for purity evaluation. The purities obtained from these two electrochemical techniques are evaluated by comparison with the microbiological autoturbidimetric and automated iodometric assays. Finally, the effect of impurities on each assay technique is presented. Electrochemical techniques were found to be less affected by impurities than other assay techniques. The use of NMR, TLC, GC, and polarography to monitor the types and levels of impurities also is discussed.

EXPERIMENTAL

Apparatus—Polarograms were recorded using a three-electrode polarograph[1]. Either a natural drop time dropping mercury electrode or a mechanically controlled drop time of 1 sec was used. Potential scan rates were 1 or 2 mv/sec. Instantaneous currents were measured. Electronic damping was not used. The three-compartment, water-jacketed polarographic cell was thermostated at the desired temperature (20–25°). The cell compartments were separated by medium-porosity sintered-glass disks and 4% agar–potassium chloride salt bridges. A saturated calomel reference electrode (SCE) and a 1-cm^2 platinum foil counter electrode were used. Water-saturated nitrogen was used for deaeration of the sample solutions.

A potentiostat[2] with current-to-voltage converter was used for coulometry. The current-to-voltage converter output was integrated electronically using an operational amplifier circuit constructed with a differential input, low drift, chopper-stabilized operational amplifier[3]. The integrator-calibrated time constant was 10.73/sec. The coulometric cell consisted of a 40-mm (diameter) × 45-mm Pyrex weighing bottle fitted with a rubber stopper containing the appropriate cell connections.

The working electrode was a 12.6-cm^2 stirred mercury pool. A magnetic stirrer was used for agitation. A 4-cm^2 platinum foil or wire counter electrode was separated from the working electrode compartment by a medium-porosity sintered-glass disk. The saturated calomel reference electrode was double isolated from the cell through a two-compartment salt bridge having sintered-glass disks. To exclude oxygen, a continuous stream of nitrogen was bubbled through the solution from a 10-mm, medium-porosity, fritted-disk immersion filter.

NMR spectra[4] were acquired from 99.7% deuterium oxide solutions, except for IV where solubility dictated the use of deuterated dimethyl sulfoxide. Sodium 3-(trimethylsilyl)propanesulfonate was used as the zero delta standard. All spectra were obtained at the equilibrium probe temperature.

Procedure—Solutions for polarography were prepared by weighing approximately 5 mg (measured to 0.01 mg) of sample into a 25-ml volumetric flask, dissolving in water, adding buffer, and diluting to volume. No surfactants were used for analytical investigations. The pH of this solution was measured. An aliquot was transferred to the polarographic cell and deaerated, and the polarogram was recorded. The diffusion current, i_d, and half-wave potentials, $E_{1/2}$, were measured by the usual procedures (6).

Coulometric solutions were prepared in deionized water at a nominal concentration of 5 mg/ml. Aliquots (5 ml) were pipetted into the coulometric cell containing the supporting electrolyte preelectrolyzed at the electrolysis potential. The number of coulombs consumed during electrolysis was determined using the calibrated time constant of the integrator, its output voltage (measured to 1 mv), and the current range of the potentiostat.

[1] Beckman Instruments Electroscan 30 operated with a natural drop time dropping mercury electrode, or Princeton Applied Research Corp. model 174A polarograph with a mechanically controlled 1-sec drop time operated in the "sampled dc" mode.

[2] Princeton Applied Research Corp. model 173 with model 176 current-to-voltage converter.

[3] Burr Brown model 3355.

[4] Varian T-60A spectrometer.

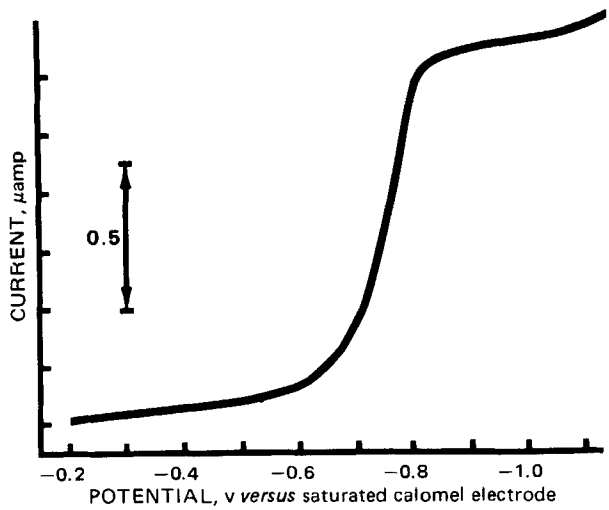


Figure 1—*Polarogram of 0.4 m*M *cefamandole nafate in pH 2.3 McIlvaine buffer.*

The total number of coulombs was corrected for the residual current observed after the electrolysis by integration of the residual current. This residual current was higher in the presence of the electrolysis products than in the preelectrolyzed electrolyte. The purity was calculated by comparison to the theoretical charge that should have been transferred for the amount of sample. These calculations were performed using:

$$Q\text{ (observed)} = RC \times V\text{ (out)} \times \text{sensitivity} \quad \text{(Eq. 1)}$$

$$Q\text{ (net)} = Q\text{ (observed)} - Q\text{ (correction)} \quad \text{(Eq. 2)}$$

$$Q\text{ (theory)} = \text{sample weight, mg} \times n \times F/\text{MW} \quad \text{(Eq. 3)}$$

$$\text{purity, \%} = Q\text{ (net)} \times 100/Q\text{ (theory)} \quad \text{(Eq. 4)}$$

where Q is charge in millicoulombs; RC is the integrator time constant per second; V (out) is integrator output voltage; sensitivity is the current-to-voltage sensitivity in milliamperes per volt; n is the number of electrons transferred per species; F is Faraday's constant, 96,500 mcoulombs/mEq; and MW is molecular weight in milligrams per mmole. Analytical results were obtained using an electrode potential of −0.875 v *versus* the saturated calomel reference electrode.

Electrochemical buffer solutions were prepared using reagent grade chemicals and were checked for contamination from electroactive impurities in the potential region of interest. Phosphate–citrate–potassium chloride 0.5 *M* constant ionic strength buffers were used unless otherwise stated.

The best NMR results were obtained when special attention was given to instrument adjustment. Optimization of spin rate, spectrum amplitude, and phasing was crucial. A spin rate of 45–50 rps prevented interference from spinning side bands. For best precision, the α-phenylmethine resonance amplitude was adjusted to full scale. Proper phasing was necessary for accurate measurements of peak heights. Spectra were acquired on 200-mg/ml solutions.

Thin-layer chromatograms were obtained using a 250-μg sample loading from an aqueous solution on silica gel plates[5]. The developing solvent was ethyl acetate–acetone–acetic acid–water (5:2:1:1). Chromatographic zones were examined by either shortwave UV light (254 nm) or after exposure to iodine vapors.

RESULTS

Polarography—Cefamandole and cefamandole nafate each gave a two-electron reduction wave at the dropping mercury electrode (Fig. 1). The transfer of two electrons was apparent from the coulometric results and by comparison of the observed diffusion current with that observed from compounds having known two-electron reductions (7). The polarographic wave corresponded to the reductive cleavage of the thioether linkage at position 3 of the cephalosporin (7). It exhibited a maximum of the first kind (8) at concentrations greater than 0.2 m*M* when the capillary drop time was 1 sec. When surfactant[6] or surface-active impurities were present, the maximum was suppressed.

[5] Merck precoated 0.25-mm silica gel 60 F-254 TLC plates.

[6] Triton X-100.

Table I—Concentration and pH Dependence of Polarographic Waves[a]

Cefamandole				Cefamandole Nafate			
pH	Concentration, m*M*	$E_{1/2}$, v *versus* SCE	i_d/C^b, μamp/m*M*	pH	Concentration, m*M*	$E_{1/2}$, v *versus* SCE	i_d/C^b, μamp/m*M*
0.67[c]	0.395	−0.749	4.15	0.60[c]	0.336	−0.744	3.98
1.60[c]	0.370	−0.776	4.03	1.66[c]	0.340	−0.769	4.01
2.29	0.202	−0.716	3.94	2.43	0.174	−0.698	3.88
2.29	0.387	−0.755	3.92	2.43	0.351	−0.750	3.85
2.29	0.395	−0.755	3.98	2.43	0.375	−0.744	3.89
2.29	0.772	−0.802	4.17	2.43	0.7079	−0.786	3.99
2.29	1.942	−0.859	4.01	2.43	1.136	−0.823	4.08
2.29	4.10	−0.902	3.99	2.43	1.676	−0.851	3.96
2.29	7.90	−0.959	4.00	2.43	3.572	−0.900	3.90
3.30	0.364	−0.798	3.92	3.31	0.353	−0.792	3.86
4.72	0.347	−0.880	3.96	4.49	0.347	−0.860	4.09
5.57[d]	0.377	−0.971	3.98	5.38[d]	0.340	−0.951	3.80
7.19	0.419	−1.122	4.58	6.91	0.344	−1.092	4.72

[a] Data were obtained using a capillary with $m^{2/3}\,t^{1/6}$ of 1.382, open circuit, 1 *M* KCl, 1-sec mechanically controlled drop time. All supporting electrolytes were 0.5 *M* constant ionic strength McIlvaine buffers except as noted. [b] i_d = 3.991 (±0.010 *SD*) *C* + 0.0234 (±0.034 *SD*) and 3.902 (±0.031 *SD*) *C* + 0.058 (±0.052 *SD*) least-squares calculation for data obtained in pH 2.3 buffer. Instantaneous currents measured at the half-wave potential are reported. [c] Hydrochloric acid, 0.32 and 0.2 *M* ionic strength for pH 0.67 and 1.60, respectively. [d] Acetic acid—acetate, 0.7 *M* ionic strength.

Plots of $\log i/(i_d - i)$ *versus* *E* of the polarograms were nonlinear at all concentrations investigated. At very low concentrations such as 2×10^{-5} *M*, the logarithmic plots became nearly linear. The limiting current was proportional to concentration and to the square root of the corrected mercury column height. It had a temperature coefficient of approximately +0.8%/degree at 25°. These observations indicate that the limiting current was diffusion controlled.

The half-wave potentials of cefamandole and cefamandole nafate were complex functions of pH and concentration (Table I), as was previously observed for related compounds (7, 9). In the pH 0.6–2.3 range, $E_{1/2}$ was generally independent of pH. In contrast, a shift of $E_{1/2}$ with pH was observed from pH 2.3 to 7. The carboxylate pKa of these compounds was about 2.6–2.9 as determined by aqueous titration or 3.0 as determined by spectrophotometry. The diffusion current normalized with respect to concentration was approximately independent of pH at or below pH 5.8 and nearly equal for both compounds (diffusion current constant, *I*, equals 2.89). The half-wave potential was dependent upon concentration. This variation is reported in Table I, but it was impossible to measure "true" values of $E_{1/2}$ when maxima were present (concentrations above 0.2 m*M*).

The precision obtained for the normalized diffusion current in the pH 2.3 supporting electrolyte for individually weighed samples of identical material was 1.1% (n = 12). Thus, for the comparison of a sample to a standard reference material, a relative standard deviation of 1.6% may be expected. Decreases in the diffusion current of approximately 0.4%/hr (3.1% in 7 hr and 9.2% in 24 hr) were observed. Therefore, all polarograms were acquired on freshly prepared solutions.

Compounds III–VIII were not polarographically reducible under the assay conditions. However, 1-methyl-5-mercapto-1,2,3,4-tetrazole sodium salt (VII) can be oxidized at either platinum or mercury electrodes. At the platinum electrode, the oxidation product of thiols is the disulfide. However, the oxidation product of thiols at a mercury electrode is the mercurous salt (10).

Table II—Comparison of Purity Assays[a]

	Cefamandole Sodium			Cefamandole Nafate		
Assay Ratio	n	Result	*SD*	n	Result	*SD*
Polarography—microbiological	4	0.999	0.022	32	1.018	0.035
Polarography—iodometric	4	1.002	0.012	34	0.991	0.029
Coulometry—microbiological	7	1.003	0.004	15	1.045	0.034
Coulometry—iodometric	6	0.989	0.006	9	0.990	0.026
Coulometry—polarography	19	1.006	0.022	7	0.974	0.038

[a] See text for explanation of assay procedures.

Comparison of Polarographic with Microbiological and Iodometric Assays—The relative purities of raw material lots of cefamandole sodium and cefamandole nafate determined by the polarographic, microbiological, and iodometric techniques are compared in Table II. All assays used a common cefamandole reference material. The polarographic assay was performed as already described. The microbiological turbidimetric assay measured the inhibition of growth of *Staphylococcus aureus* (ATCC 9144). The assay precision is about 2–4% as measured by relative standard deviation. The automated iodometric titration measured the increase in iodine uptake by the β-lactam following alkaline hydrolysis (11). The relative standard deviation for this assay is 1–2%.

The polarographic purity results were in excellent agreement with the purities determined by the other assay techniques. The observed ratio of assay results was less than 1 *SD* from unity for both cefamandole and cefamandole nafate (Table II).

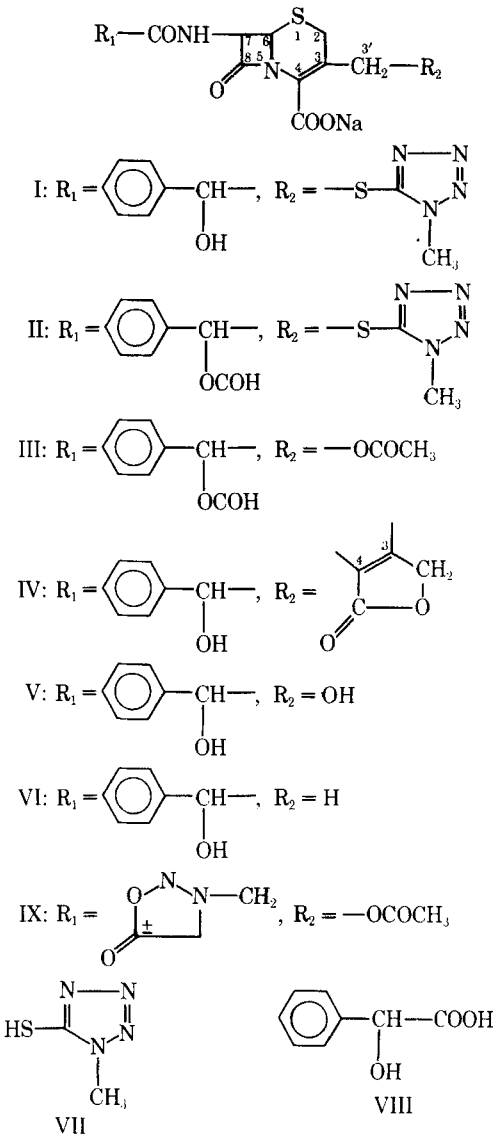
Controlled Potential Coulometry—When the anode and cathode compartments of the coulometric cell were separated, a two-electron reductive cleavage of the thioether bond was obtained. This arrangement corresponded to the normal experimental one. If the anode were located in the main portion of the cell, then the 1-methyl-5-mercapto-1,2,3,4-tetrazole formed at the mercury pool cathode would be transported to the platinum anode and oxidized to the corresponding disulfide. Under these conditions, a net three-electron transfer would be observed.

Analytical data were obtained using a cell with separated anode and cathode compartments. Plots of the logarithm of the current *versus* time were not linear, indicating that the reduction was not a simple electron transfer. This result was consistent with previous observations (7).

The products of the controlled potential coulometric reduction of cefamandole were examined by TLC and polarography. TLC indicated the presence of two major, one minor, and several trace zones. The retention factors for the major zones corresponded to the tetrazole derivative (VII) and VI. The minor zone retention factor correlated with 7-D-mandelamidocephalosporanic acid (mandelic acid form of III). The only identified trace zone appeared to be V. The amount of free VII as measured by polarography was greater than 80% of the amount expected for quantitative formation of this compound.

Comparison of Coulometry with Other Assay Techniques—The relative purities obtained for raw material lots of cefamandole and cefamandole nafate using the coulometric, microbiological, and iodometric assays are given in Table II. A comparison of the coulometric and polarographic assays is included also.

The coulometric assay results were in good agreement with results obtained by the other techniques. However, the ratio of the coulometric assay to the microbiological assay was more than 1 *SD* from unity for cefamandole nafate. This result may be explained by the relatively small number of samples from which the comparison was made. Moreover, the difference probably partially resulted from the nature of the microbiological assay for cefamandole nafate. For this assay, the sample is hydrolyzed to cefamandole in a sodium carbonate solution at room temperature and then is compared to a standard of cefamandole. In contrast, the polarographic and iodometric assays of cefamandole nafate use a standard lot of cefamandole nafate for the comparison. Thus, the mi-



crobiological assay for cefamandole nafate uses a different reference material and a more complicated procedure than do the chemical assays. The coulometric assay does not need a reference material.

NMR—Spectra of I–V are shown in Fig. 2. The spectrum of IV obtained in dimethyl sulfoxide exhibits the expected solvent shifts when compared to the other compounds. The assigned chemical shift values are listed in Table III.

Significant spectral differences occurred between compounds in the areas of δ 5.2–6.2 and 2.05. The α-phenylmethine resonance shifts from the region of δ 5.25 in mandelic acid derivatives to the δ 6.2 region in compounds containing the formyl ester of mandelic acid. This shift

Table III—60-MHz NMR Chemical Shift Values (δ)

Proton	Compound I	II	III	IV[a]	V	VI	VIII
α-Mandeloyl-formyl	—	8.33	8.28	—	—	—	—
Phenyl	7.45	7.43	7.50	7.37	7.45	7.47	7.52
α-Phenylmethine	5.25	6.20	6.20	5.08	5.25	5.25	5.37
C-7	5.60	5.63	5.63	5.83	5.60	5.55	—
C-6	5.05	4.97	4.95	5.08	5.07	5.00	—
3′-Methylene	4.17	4.17	4.83	5.03	4.22	1.92	—
N-Methyl	3.97	3.95	—	—	—	—	—
C-2	3.50	3.40	3.28	3.75	3.45	3.38	—
3′-Acetoxy	—	—	2.07	—	—	—	—

[a] Deuteratated dimethyl sulfoxide.

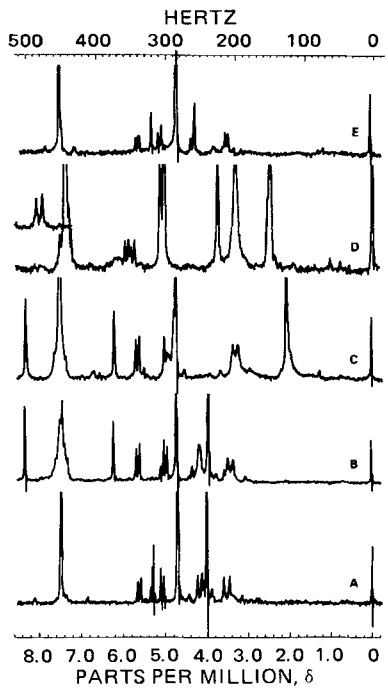


Figure 2—*The 60-MHz NMR spectra of (A) I, (B) II, (C) III, (D) IV (in dimethyl sulfoxide), and (E) V.*

provides a convenient means of detecting substances containing the mandelic acid functionality (*e.g.*, I and IV–VI) in cefamandole nafate. Accurate measurements for the amounts of these compounds in cefamandole nafate were possible by comparing the height of the δ 5.25 peak to the sum of the heights of the peaks at δ 5.25 and 6.2. The detection level was 2 mole %, and a linear calibration curve was obtained for samples spiked with known amounts of impurities. The slope of the calibration curve was unity when the observed ratio was plotted *versus* mole percent added.

Cefamandole and cefamandole nafate had no resonances upfield of δ 2.5. Trace solvent peaks were occasionally observed such as acetone at δ 2.2 and acetates at δ 2.0. However, impurities containing an acetoxy function such as III had a very sharp resonance at δ 2.05. This resonance provided a positive indication of their presence. A quantitative measure of the mole ratio of these compounds was obtained by comparison of the acetoxy peak height to the α-phenylmethine peak height (δ 6.2). The detection level was about 1 mole % of acetoxy compounds, and the calibration curve was linear with a slope of one when the acetoxy peak height was normalized to one proton.

TLC—TLC was used to identify impurities, degradation products, and the products of the electrochemical reduction. It also provided a rough estimate of the levels of these substances. The relative retention factors for this series of compounds are given in Table IV. With a 250-μg plate loading, generally 1% of a substance was visible on the developed plate. However, D-mandelic acid was not visible except above the 10% impurity level.

DISCUSSION

The initial description of the electrochemistry of cephalosporins was given by Jones *et al.* (9). Those investigators (9) and Hall (7) used the

Table IV—TLC Data

Compound	R_f	Detection Limit[a], %
I	0.46	1
II	0.52	—
III	0.58	1
IV	0.82	1
V	0.34	1
VI	0.66	1
VII	0.92	1
VIII	0.74	~10

[a] Detection limit using a 250-μg sample of cefamandole nafate.

polarographic reduction wave for quantitative assays. Controlled potential electrolysis has been used to obtain n values (7) and for electrosynthesis (12) but not for quantitative analysis. We found that, under appropriate conditions, cefamandole and cefamandole nafate may be quantitatively reduced at a mercury pool electrode at a controlled potential.

Hall (7) was able to identify explicitly the functional group that undergoes reduction in cephaloridine, cephalothin, cephalosporin C, and IX, a cephalosporin similar to cefamandole. In all cases, the substituent at the 3-position was reductively eliminated in a two-electron, irreversible polarographic wave. The initial reduction products probably were the free, protonated 3-position substituent and the anion radical of the cephalosporin nucleus. In aqueous solutions, the anion radical immediately reacted with a proton donor such as the hydronium ion or the supporting electrolyte components. Typical products reported (7, 12) were the 3-methylenecepham derivatives, which were easily isomerized to the corresponding 3-deacetoxycephalosporanic acid derivatives such as VI[7]. These observations are consistent with the partial identification of the coulometric reduction products described.

The shift of the polarographic half-wave potential observed with pH indicated involvement of hydronium ions in the reduction mechanism. This potential shift is between 50 and 90 mv cathodic per pH unit but is apparently not linear with pH.

Cyclic voltammetry was used to elucidate the mechanism of this reaction. The qualitative features—no anodic peak at scan rates up to 5 v/sec, approximately constant current function (peak current/square root of scan rate) at low scan rates, and a cathodic shift of reduction peak potentials with increasing scan rate—were in agreement with a chemical reaction following charge transfer (13). However, the large effects due to adsorption of impurities, uncompensated potential drops, and variation of parameters with concentration prevented any detailed correlation of experimental results with theoretical expectations. It can, however, be assumed that the 1-methyl-5-mercapto-1,2,3,4-tetrazole is eliminated and then protonated. Simultaneously, the cephalosporin nucleus moiety undergoes successive chemical reactions, probably involving the solvent. Thus, the products of the electrochemical reaction are quickly removed and unavailable for an electrochemical reoxidation step except at extremely short times. These chemical reactions account for the irreversible electrochemical reduction observed on the experimentally accessible time scale.

The data presented indicate that polarography and controlled potential coulometry are useful assay techniques for purity determinations on raw material samples of cefamandole and cefamandole nafate. The electrochemical assay results were essentially identical to the microbiological and iodometric assay results. However, a good analytical assay also must be selective or essentially free from interferences. The various assay techniques were examined to determine what effect impurities or degradation products had on each technique.

The most likely impurity in cefamandole nafate is III. This impurity occurs when the acetoxy group is not displaced by the substituted tetrazole in the synthetic sequence. In solution, cefamandole nafate is hydrolyzed by an alkaline-catalyzed reaction (5). Cefamandole nafate and any cefamandole formed by hydrolysis respond essentially identically in the chemical assay procedures. The degradation of cefamandole or cefamandole nafate then follows one of two possible sequences according to the pH of its environment. These sequences were reviewed for a general cephalosporin (7) and studied for these particular compounds[8].

In acidic media, cefamandole initially loses the substituted tetrazole to give the hydroxymethyl compound, V, and VII. Compound V rapidly dehydrates to form the α,β-unsaturated lactone, IV. Under extremely harsh conditions, mandelic acid (VIII) is cleaved and the cephalosporin nucleus is destroyed. In basic media, the substituted tetrazole is initially lost also. At prolonged times or high temperatures in basic media, the mandelic acid substituent is cleaved and the β-lactam ring is opened[8]. Therefore, III–V, VII, and VIII can be present in aged cefamandole nafate as either initial impurities or degradation products. The polarographic assay for cefamandole or cefamandole nafate, which measures the thioether linkage, will not have interference from these compounds and can be considered a selective assay technique.

In the microbiological assay, any compound that inhibited the growth of the test organism was a positive assay interference. In general, compounds with an intact cephalosporin nucleus exhibited some activity. Compounds III–V interfered with the microbiological assay. Compound III had the highest activity of these interferences. The iodometric assay is based on the reaction at the β-lactam. The likely impurities and degradation products III–V interfered with this assay.

Even though the electrochemical assays were very selective, the presence of large amounts of impurities, especially if they were surface active, tended to distort the polarographic wave and make it irreproducible. This problem was not a major concern until the contaminant content was more than 20–30%. In controlled potential coulometry, none of the known contaminants interfered directly. At high levels of impurity (10–20%), a decrease in precision was noted. The greatest value of the coulometric technique was in the evaluation of relatively pure raw materials such as the establishment of an analytical reference standard material. This reference material was then used to assay material by a precise, selective technique such as polarography.

In addition to a selective, precise assay, one needs a thorough screen for impurities and methods of quantitating detected contaminants. NMR, TLC, GC, and polarography are useful. The use of NMR for the initial evaluation of impurities and for monitoring the extent of degradation was discussed. TLC was used to detect the presence of cefamandole and III–V and VII in cefamandole nafate with an approximate sensitivity level of 1–3%. Mandelic acid may be determined by a separate GC assay if necessary. The free substituted tetrazole (VII) can be measured using the polarographic oxidation wave.

SUMMARY

Both cefamandole and its formyl ester, cefamandole nafate, can be assayed by two electrochemical methods. Dropping mercury electrode polarography was used to determine the purity of the samples by comparison to a reference material of known purity. Controlled potential coulometry was used as an absolute measure of the purity of these compounds without the need for a reference material. These techniques utilize reductive cleavage of the thiotetrazole substituent at the 3-position.

With pure raw material, the results from the electrochemical assays are essentially identical to the purity results obtained with the microbiological autoturbidimetric and automated iodometric assays. The precision of the electrochemical assays is better than the microbiological assay and comparable to the iodometric assay.

For raw material samples containing typical impurities or degradation products, polarography is the most selective of the three types of purity assays. NMR, TLC, GC, and polarography are used for the detection and quantitative measurement of compounds that could interfere with the various assay procedures.

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Electron-Capture GLC Determination of Nanogram to Picogram Amounts of Isosorbide Dinitrate

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Abstract □ A GLC method for the determination of plasma isosorbide dinitrate using electron-capture detection is described. The organic nitrates are especially suited for electron-capture detection if the detector temperature is optimized for maximum sensitivity, *e.g.*, 175°. Proper maintenance of the detector and column assures reproducible data in the low nanogram range. The extraction procedure described is simple, efficient, and expedient for processing large numbers of samples. The method was used to study plasma levels in four human volunteers after a single dose of a 5-mg chewable isosorbide dinitrate tablet. Concentration levels of isosorbide dinitrate as low as 0.5 ng/ml of plasma can be measured by this procedure.

Keyphrases □ Isosorbide dinitrate—electron-capture GLC analysis, human plasma □ GLC, electron capture—analysis, isosorbide dinitrate, human plasma □ Vasodilators, coronary—isosorbide dinitrate, electron-capture GLC analysis, human plasma

The low dosage of isosorbide dinitrate sufficient for pharmacological effectiveness results in low circulating blood levels of the drug, and difficulties exist in assaying for low nanogram to picogram amounts. Previously described GLC methods (1, 2) were either laborious or not sensitive enough to follow rapidly declining blood levels of isosorbide dinitrate.

Since only limited data are available on the blood levels of isosorbide dinitrate following administration of therapeutic doses, a method was needed that would allow reliable estimations of blood isosorbide dinitrate levels in the low nanogram to picogram range using conventional electron-capture GLC instrumentation. This report describes an assay method for isosorbide dinitrate that is expedient, sensitive, and reproducible for routinely handling large numbers of samples.

EXPERIMENTAL

Reagents and Materials—Benzene[1] and ethyl acetate[1] were glass-distilled reagent grade solvents. Anhydrous sodium sulfate[2] was reagent grade, ACS certified. Charcoal-treated paper disks[3], 6.5-mm diameter, were made with a paper punch-out.

One hundred grams of anhydrous sodium sulfate powder was washed with benzene for 20 min, allowed to air dry, and then placed in an oven at 100° until needed.

The charcoal-treated paper disks were similarly washed in benzene. Excess benzene was removed, and the paper disks were allowed to air dry. The disks were stored in an air-tight container until required.

Isosorbide dinitrate[4] and isoidide dinitrate[4] were purified by recrystallization from ethanol.

Plasma Level Study—Isosorbide dinitrate was administered to four healthy volunteers (one male, age 34; three females, ages 23, 25, and 27); each received a 5-mg chewable isosorbide dinitrate tablet[5]. Blood, 10 ml, was drawn from the cubital vein into a heparinized tube from each subject 0, 15, 30, 60, 120, and 180 min following isosorbide dinitrate administration. The blood was immediately centrifuged at 3000 rpm in an angular head clinical centrifuge[6] for 15 min. The plasma was collected and stored at −10° prior to analysis.

Extraction of Isosorbide Dinitrate—Five-milliliter aliquots of benzene were added to a 15.0-ml graduated conical centrifuge tube containing 1.0 ml of plasma spiked with 10 ng of isoidide dinitrate as an internal standard. The tube was mixed on a vortex[7] for 1 min and centrifuged at 2000 rpm to separate the phases. Then the benzene phase was carefully removed to another 15-ml centrifuge tube. A 0.5-g portion of benzene-washed sodium sulfate and three benzene-washed charcoal-treated paper disks were added to the benzene extract.

The tube and its contents were again vortexed for 15 sec and centrifuged as previously described. As much of the benzene layer as possible was carefully removed to another centrifuge tube and dried completely under a nitrogen stream (using an in-line activated silica gel desiccant filter). To each residue was added 100 μl of ethyl acetate just prior to injection. Samples to be analyzed at a later time, *i.e.*, up to 24 hr, were stored, covered tightly, and protected from light.

Electron-Capture GLC Detection—The prepared GLC samples were analyzed using a gas chromatograph[8] equipped with a ^{63}Ni-electron-capture detector. A glass column (120 cm × 4 mm i.d.) was packed with 3% QF-1 on Gas Chrom Q, 100–200 mesh. The injection port and oven temperatures were maintained at 210 and 150°, respectively. The detector was set at 175° and operated with a pulse interval of 150 μsec. The flow rate of the carrier gas, argon with 5% methane, was 95 ml/min. Under these conditions, the retention times of isoidide dinitrate and isosorbide dinitrate were 2.0 and 3.0 min, respectively. The injection volume was 5 μl, and the injection was performed in duplicate.

Calculations—A stock solution of isosorbide dinitrate in absolute ethyl alcohol (1 μg/ml) was used to spike plasma in the 5–75-ng/ml range. A stock solution of isosorbide dinitrate (0.5 μg/ml) was used to spike plasma in the 0–5-ng/ml range. The samples were likewise spiked with 10 ng of isoidide dinitrate as an internal standard. A response factor (peak area ratio) was calculated for the standard. The concentration of isosorbide dinitrate in the samples was determined from the standard's response factor.

Extraction Efficiency—The overall recovery of isosorbide dinitrate by the described extraction procedure was determined by spiking plasma

[1] Burdick-Jackson Labs., Bodman Chemicals, Narberth, Pa.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] S & S charcoal-treated filter paper No. 505.
[4] Stuart Pharmaceutical Division, ICI United States Inc.
[5] Sorbitrate, Stuart Pharmaceutical Division, ICI United States Inc.
[6] International Equipment Co., Needham, Pa.
[7] Vortex Genie, Fisher Scientific.
[8] Hewlett-Packard HP 7620A.