

Polarographic Determination of Ethacrynic Acid

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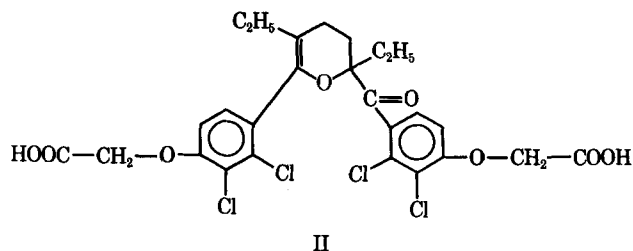
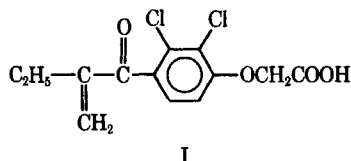
Abstract □ Polarography was applied to the analysis of ethacrynic acid in the presence of its principal degradation product, a dimer. The application of the polarographic assay to dosage forms is discussed, and some stability data are given.

Keyphrases □ Ethacrynic acid—polarographic assay, dosage forms □ Polarography—analysis, ethacrynic acid

Ethacrynic acid (I)¹ is a potent saluretic agent, chemically unrelated to previous diuretic agents. The chemical synthesis and biological properties of ethacrynic acid and a number of its homologs and analogs are given elsewhere (1). These studies established that the α,β -unsaturated aryl ketone moiety was essential for optimum biological activity. Any alteration of this grouping, such as saturation of the double bond or reduction of the ketone to the corresponding alcohol, resulted in a marked diminution in diuretic activity. The α,β -unsaturated ketone group is quite reactive chemically. A number of agents such as sodium bisulfite, mercaptans, and bromine add rapidly across the double bond. In addition, ethacrynic acid undergoes a Diels-Alder type of condensation to produce a dimer (II) (2).

Dimerization occurs under a variety of conditions and is the principal degradative pathway for the compound in pharmaceutical dosage forms. This finding led to a search for assay procedures that would distinguish between intact ethacrynic acid and the dimer. The usual bromination assay proved unsuitable, since the reactive double bond of the dimer takes up bromine quantitatively. The UV absorbances of ethacrynic acid (λ_{\max} , 271 nm., $A_{1\%}^{1\text{cm}}$, 117 in CH_3OH) and the dimer (λ_{\max} , 261 nm., $A_{1\%}^{1\text{cm}}$, 127) were not sufficiently different to distinguish between the compounds in a stability-indicating assay procedure.

A procedure was devised for direct measurement of II in the presence of ethacrynic acid based on the reaction of ethacrynic acid with excess sodium bisulfite (by addition across the double bond in neutral aqueous solution) to form a water-soluble nonextractable adduct while II remained unreacted (3). Acidification of the neutral solution, followed by extraction with benzene, removed II, leaving the polar ethacrynic acid-bisulfite adduct in the acidic aqueous layer. The separated dimer was then



measured by UV analysis. Other compounds, which either do not react with sodium bisulfite or are extractable under these conditions, accompany the dimer in the benzene layer.

On the assumption that ethacrynic acid would be reducible at the dropping mercury electrode (DME), polarography was tried as an assay procedure. Preliminary experiments revealed that it gave well-defined polarographic steps which could be used to assay for intact ethacrynic acid in the presence of II. The polarographic assay procedure developed was successfully applied to the analysis of the solid, as well as its pharmaceutical dosage forms, and some of the data obtained are given in this publication.

EXPERIMENTAL

Equipment and Reagents²—Polarographic measurements were carried out with an electrochemograph³. A standard H-cell was used containing a saturated calomel electrode (SCE) separated from the sample compartment by an agar plug (3% agar dispersion prepared with saturated KCl solution) and a fritted-glass diaphragm. The DME was employed at a drop time of 2.5 sec., and at an ethacrynic acid concentration of 0.2 mg./ml. in the supporting electrolyte described below, a wave height of 4.2 μ amp. was obtained for the ethacrynic acid step.

A 0.50 *M*, pH 8.0, phosphate buffer, used as part of the supporting electrolyte, was prepared as follows. Dissolve 68.1 g. of KH_2PO_4 in about 400 ml. of purified water. Add a sufficient quantity of 1.0 *N* sodium hydroxide to adjust the pH to 8.0 and dilute to 1000 ml. with purified water. Other components of the supporting electrolyte were Emulphor EL-620⁴ (maximum suppressor), used as a 0.1% stock solution in purified water, and alcohol USP. All chemicals employed were the best grade available and were not purified further.

Polarographic Procedure—For ethacrynic acid tablets, accurately weigh a portion of finely powdered tablet sample, equivalent to about 100 mg. of ethacrynic acid, into a 50-ml. volumetric flask. Add about 35 ml. of alcohol and mechanically agitate the flask for about 30 min.; then dilute to the mark with alcohol. Centrifuge a portion of the dispersion and pipet 5 ml. of the clear supernatant into a second 50-ml. volumetric flask containing 25 ml. of phosphate buffer and 1 ml. of the Emulphor EL-620 solution. Dilute to the mark with purified water and mix well. Transfer a portion of this solution to the H-cell and purge with nitrogen. At the end of the nitrogen purge, insert the DME, direct the nitrogen stream over the top of the solutions, and record the polarogram from -1.0 v.

² The ethacrynic acid, dimer, and other compounds discussed here were synthesized and characterized at the Merck Sharp & Dohme Research Laboratories, West Point, Pa., and Rahway, N. J.

³ Leeds and Northrup model E.

⁴ Antara Chemicals, Division of GAF, New York, N. Y.

¹ Edecrin, Merck Sharp & Dohme, West Point, Pa.

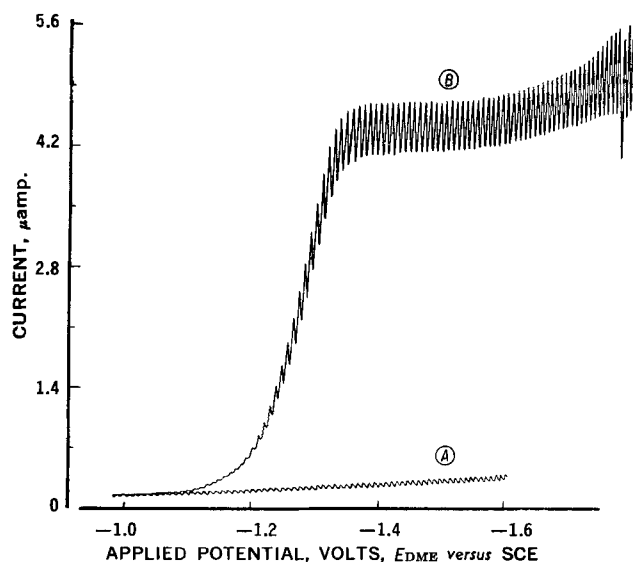


Figure 1—Ethacrynic acid polarogram in pH 8.0 phosphate buffer containing 10% alcohol. Key: A, blank; and B, 0.2 mg./ml. of ethacrynic acid.

versus the SCE to -1.8 v. versus the SCE. Prepare both a blank solution and a standard solution (0.2 mg. ethacrynic acid/ml.), using a standard reference lot of ethacrynic acid, in the same supporting electrolyte as employed for the test sample. Obtain the polarograms for the blank and standard solutions in the same way.

For sodium ethacrylate for injection, reconstitute a vial of 1 ml. of purified water and dilute the contents to 25 ml. with alcohol. Pipet 5 ml. of the reconstituted solution into a 50-ml. volumetric flask and proceed as described for the tablet assay starting with: "containing 25 ml. of phosphate buffer. . ."

Calculations—Take the net step height of the recorded polarograms as the difference between the step height of the standard or sample solution at -1.45 v. versus the SCE and the height exhibited by the blank solution at -1.45 v. versus the SCE. Use the average of the recorded pen excursions for computing the step height if damping is employed:

$$\text{mg. found} = \frac{S_1}{S_2} \times \text{mg. standard} \times \text{dilution factor} \quad (\text{Eq. 1})$$

where mg. found = milligrams of ethacrynic acid found in portion of sample taken for analysis, S_1 = net step height of sample solution, S_2 = net step height of standard solution, and mg. standard = milligrams of standard taken for analysis.

RESULTS AND DISCUSSION

General Polarographic Behavior—Ethacrynic acid exhibits a well-defined polarographic step at the DME in pH 8.0 buffer containing 10% alcohol, with an $E_{1/2}$ of -1.28 v. versus the SCE (Fig. 1). The $E_{1/2}$ is pH dependent, shifting to more negative potential with increasing pH. At a pH below 7, the single wave is split into two whose total height is about the same height as the single wave exhibited at a higher pH. A second, more negative, less well-defined step is also observed for ethacrynic acid at pH 8.0, with an $E_{1/2}$ of -1.75 v. The height of the second step is about one-tenth the height of the first step. The dimer (II) gives a poorly defined polarographic step, with an $E_{1/2}$ of -1.68 v. versus the SCE, which does not interfere with the first ethacrynic acid step (Fig. 2).

The height of the first ethacrynic acid step is proportional to the concentration over a fairly wide range. No deviation from linearity for a calibration curve was noted for solutions up to about 2×10^{-3} mM in ethacrynic acid.

Specificity of Polarographic Assay—As previously indicated, II does not interfere with the ethacrynic acid step (Fig. 2). Dihydro-ethacrynic acid, the bisulfite-ethacrynic acid adduct, the cysteine-ethacrynic acid adduct, and several other ethacrynic acid reaction products, in which the double bond of the α,β -unsaturated ketone

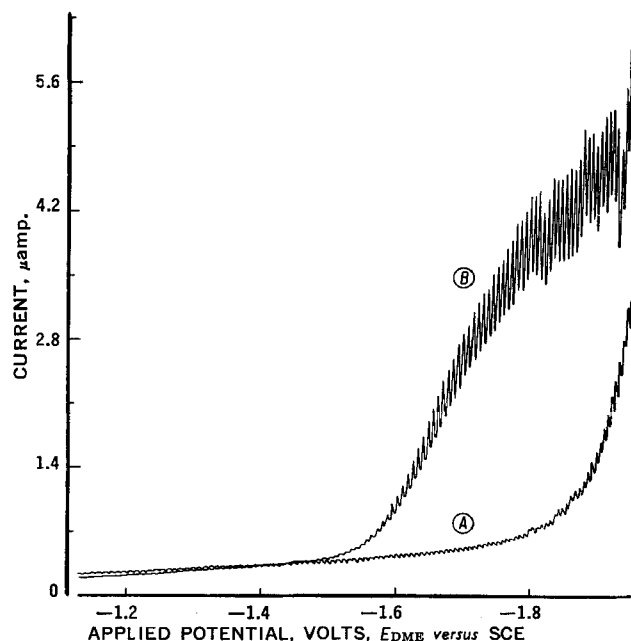


Figure 2—Dimer (II) polarogram in pH 8.0 phosphate buffer containing 10% alcohol. Key: A, blank; and B, 0.2 mg./ml. of the dimer (II).

moiety was "saturated," likewise did not interfere with the ethacrynic acid step.

Precision Studies—The relative standard deviation for the mean of duplicates for both ethacrynic acid tablet assays and sodium ethacrylate intravenous assays was within $\pm 2\%$ based on 6 months of experience with the assays in several laboratories.

Interference Studies—None of the excipients employed in the ethacrynic acid formulations was found to interfere with the assay at the ethacrynic acid dilution normally used (final concentration of 0.2 mg./ml.).

Stability of Ethacrynic Acid in Dosage Forms—No significant amount of degradation was noted for ethacrynic acid in both dosage forms stored for up to 36 months at room temperature. Some loss of intact ethacrynic acid was noted by polarography in samples stored at 37° for 30 months. The thermal instability of crystalline ethacrynic acid at elevated temperatures was demonstrated by heating the compound alone at 105° for 44 hr. About 30% intact ethacrynic acid was found (Fig. 3). By way of contrast, crystalline ethacrynic acid samples that had been stored in screw-capped, glass bottles at

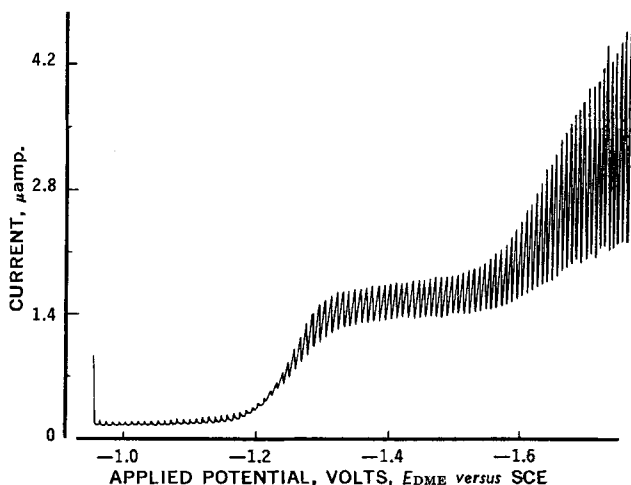


Figure 3—Ethacrynic acid polarogram after storage of solid for 44 hr. at 105° . Wave height shown is about 30% of expected for intact ethacrynic acid.

room temperature for almost 4 years did not exhibit any degradation.

Stability of Ethacrynic Acid in Solution—A solution of sodium ethacrylate (pH 6.8, 20 mg./ml.) showed no loss of intact ethacrynic acid after 3 days at room temperature. Losses were noted at elevated temperatures, however, indicating that sterile solutions of ethacrynic acid should be prepared by suitable filtration rather than by autoclaving.

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TECHNICAL ARTICLES

Effects of Moisture in Compaction of Particulate Material

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Abstract □ By using an ideal particulate system of crystalline sodium chloride and three liquids—water, decahydronaphthalene, and light liquid paraffin—the role of moisture in the compaction process was investigated. Interrelated effects of liquid at the die wall boundary and within the compact are discussed. Although differences in the behavior of the three liquids could be attributed partly to the viscosity difference, water apparently exerted a boundary lubricant effect in addition to hydrodynamic properties. Despite its lower viscosity, water was, therefore, a more effective lubricant than light liquid paraffin. In general, decahydronaphthalene adversely affected the compaction process. Cumulative changes in the difference between applied and transmitted force during consecutive compressions can be used to assess the lubricant efficiency of materials.

Keyphrases □ Compaction process—role of moisture, ideal particulate system □ Compression changes—lubricant efficiency assessment, ideal particulate system □ Water as lubricant—with sodium chloride, role in compaction process □ Die wall effects—studied using ideal particulate system of sodium chloride and water, decahydronaphthalene, or paraffin

During compaction of particulate material, the proportion of applied force transmitted to the die wall is influenced by several factors including: (a) the radial component of the applied force, and (b) the effective area of contact and coefficient of friction at the die wall. Since interparticulate lubrication increases the ratio of radial stress to axial stress (1), application of lubricant to the die is more effective than the addition of lubricant to a powder before compression (2). However, lubrication of both the die wall and interparticulate junctions produces a net decrease in the die wall reaction (1), because reducing the coefficient of interparticulate

friction usually has little effect on die wall friction compared with a decrease in the coefficient of friction at the die wall.

A reduction in friction may be produced by: (a) hydrodynamic lubricants, forming a finite layer of low shear strength which prevents contact between adjacent surfaces; and (b) boundary lubricants which reduce adhesive forces between the surfaces.

The high viscosity of a boundary lubricant may produce an excessive resistance to shear (3). Therefore, a medium viscosity is often preferable, although the resistance to contact between sliding surfaces is less. Whereas values of shear strength were not directly related to lubricant efficiency, Lewis (4) found that lubricants with a lower melting point were more efficient, possibly due to the formation of a film on the die. Strickland *et al.* (5) showed that if more than 1% stearic acid was present in a sulfathiazole granulation, the semisolid material itself resisted compression. For a liquid lubricant such as mineral oil, however, increasing the concentration to 4% produced a continual decrease in the required compression force.

Numerous authors (6-8) commented on the effects of moisture during compaction. Anomalous compression results, attributed by Train (9) to electrostatic effects, were eliminated by humidifying the material. Wolff *et al.* (10) observed that a nonlubricated granulation could not be compressed when the moisture content was greater than 4%, although the results of Stephenson (11) and Shotton and Rees (8) showed that die wall friction may be reduced in the presence of moisture.