exists. Physicians have intuitively recognized the need to reduce dosages of some agents, notably certain antibiotics, in patients with renal insufficiency. Toxicologists might properly concern themselves with the need to evaluate the toxicity of new pharmacologic agents in hypoexcretory animals as the mouse preparations described here. Such evaluation might be made as a part of drug safety evaluation during the preclinical testing period.

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# Determination of Benzoyl Peroxide Stability in Pharmaceuticals

By MURRAY P. GRUBER and ROBERT W. KLEIN

**Spectrophotometric, titrimetric, and polarographic techniques were compared for stability testing of commercially available benzoyl peroxide pharmaceuticals. Initial testing revealed comparable results but significant discrepancies were observed on prolonged testing. The titrimetric procedure employed showed little degradation of the benzoyl peroxide at elevated temperatures, while both the spectrophotometric and polarographic methods demonstrated radical potency losses under the conditions of testing. Agreement was excellent between the polarographic and spectrophotometric procedures above 70 per cent of the original assay with a significant divergence being noted only below this level. A rapid, reproducible procedure of sufficient accuracy for pharmaceutical product stability and control purposes is presented.**

WITH THE INTRODUCTION of benzoyl peroxide as a topical medicinal, this laboratory began an investigation into the published methods of analysis with a view to pharmaceutical stability evaluation. Previous methods have been employed to establish the absence of residual peroxides and associated by-products in the manufacture of plastics, polymers, fats, oils, breads, and flours. Since the primary purpose of any pharmaceutical test is specificity, it was believed that a more intensive investigation of the available methods was necessary prior to the adoption of any particular technique.

Titrimetric, spectrophotometric, and polarographic methods were compared for the determination of benzoyl peroxide stability in the dry form and in commercially available pharmaceutical preparations. The titrimetric method employed in this comparison was a modification of Wheeler's (1) procedure, a variation of the technique originally proposed by Lea (2).

Many spectrophotometric procedures have been described for the determination of organic peroxides, and the relative merits and disadvantages of these techniques have been thoroughly evaluated by Kolthoff and Medalia (3) and others (4, 5). The spectrophotometric procedure employed in this comparison was a modified form of the method of Banerjee and Budke (6). They adapted the iodimetric procedure of Heaton and Uri (7), using a 2 to 1 mixture of acetic acid and chloroform, instead of the proportions suggested by Lea (2). This technique proved to be the most promising due to the reduction of any tendency toward continued autooxidation, catalyzed oxidation, and/or induced oxidation. These properties were associated with the higher polarity of the solvent system. This procedure was designed for trace quantities of peroxide, but it was found applicable, by judicious choice of sample size and dilutions, to pharmaceutical preparations containing up to 10% benzoyl peroxide, with excellent accuracy and reproducibility.

A polarographic method was utilized for comparison as a result of the work of Ricciuti, Coleman, and Willets (8), who performed a statistical evaluation of a modified Wheeler procedure compared to a polarographic method for the analysis of tetralin hydro-peroxide and found the polarographic technique to be more reliable where impure products were encountered, due to its greater specificity. The various methods for polarographic measurement of organic peroxides are closely related in procedure and similar in results; therefore, a modification of the procedure of Bezuglyi and Dmitrieva (9) was employed.

Many pharmaceutical laboratories do not have polarographic instrumentation available because of the limited applications of polarography in this field. For this reason, a rapid, accurate, and reproducible spectrophotometric method is proposed for laboratory control and stability testing of benzoyl peroxide in pharmaceutical preparations.

## EXPERIMENTAL

### Spectrophotometric Assay

**Apparatus**—Beckman model DU spectrophotometer: 1-cm. matched cells were used.

**Reagents**—Nitrogen, research grade; iodine, A.R.; glacial acetic acid, A.R.; chloroform, A.R.;

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potassium iodide solution: dissolve 50.0 Gm. of potassium iodide, A.R., in 50 ml. of freshly deionized water in a 100-ml. graduated cylinder. Dilute to 100 ml. with freshly deionized water. This solution must be made up fresh, and should be colorless.

**Procedure**—*Standard Curve Preparation*—Weigh accurately about 635 mg. of iodine and dissolve in approximately 300 ml. of 2:1 glacial acetic acid–chloroform. Care should be taken to assure complete solution of the iodine. This is best accomplished by warming the iodine in the solvent on a water bath with frequent stirring. Keep the solution covered to avoid any loss of iodine. Dilute the solution to 500.0 ml. with the solvent mixture. Pipet 0, 1-, 2-, 3-, 4-, and 5-ml. aliquots into glass-stoppered test tubes; dilute to 25.0 ml. with the 2:1 acetic acid–chloroform solution. Insert a glass capillary tube into the bottom of the test tube and purge solution for 1.5 min. with nitrogen. Add 1 ml. of the potassium iodide solution, purge for 1 min. with nitrogen, stopper, and mix. Measure the absorbance of the solution immediately at 470 mμ in a 1-cm. covered cell *versus* a 2:1 acetic acid–chloroform blank. Plot the mg. of benzoyl peroxide (121.1/126.9 × mg. iodine) *versus* the absorbance at 470 mμ.

*Sampling Procedure for Stability Assays*—This section would ordinarily not be included. However, it is the authors' opinion that other investigators may lose valuable time in setting up stability tests, only to find that the samples under consideration had irreversibly separated during storage at elevated temperatures, thereby invalidating any further exploration.

Accurately weigh a sample equivalent to approximately 50 mg. of benzoyl peroxide into a screw-top glass vial. Record the exact weight and place an identifying mark on the vial. Repeat this procedure for as many analyses as required for the entire study. Place the vials in a controlled temperature oven at the desired test temperature. Two vials should be removed from the oven at predetermined intervals for (duplicate) stability assays.

For initial assays, weigh the sample into a tared 150-ml. beaker and proceed as directed under *Sample Preparation and Analysis*, beginning with "Bring the volume of liquid . . . ."

*Sample Preparation and Analysis*—To the sample vial, add 2:1 glacial acetic acid–chloroform and carefully wash entire contents into a 150-ml. beaker. Repeat four additional times, taking care that no residue remains in vial. Bring the volume of liquid in beaker to approximately 50 ml. with the 2:1 acetic acid–chloroform. Stir for 5 min. Quantitatively transfer the solution to a 100-ml. volumetric flask, filtering through a Whatman No. 4 filter paper (previously washed with the 2:1 acetic acid–chloroform). Wash the beaker with two additional portions of the 2:1 acetic acid–chloroform, filtering these into the volumetric flask; bring to volume with same. Mix well. Pipet a 5-ml. aliquot of the above into a 50-ml. glass-stoppered test tube. Pipet in 20.0 ml. of the 2:1 acetic acid–chloroform, mix, and purge the solution with nitrogen for 1.5 min. Immediately add 1.0 ml. of the 50% w/v KI solution and purge with nitrogen for 1 min. Stopper immediately. Place in the dark for exactly 1 hr. Then rapidly read the absorbance at 470 mμ in a covered 1-cm. cell *versus* a 2:1 acetic acid–chloroform blank. Determine the weight of benzoyl peroxide from the standard curve.

$$\text{benzoyl peroxide } \%(\text{w/w}) = \frac{\text{mg. benzoyl peroxide (from curve)} \times 2}{\text{sample wt. (Gm.)}}$$

### Titration Assay

**Reagents**—Sodium iodide solution: dissolve a sufficient quantity of sodium iodide, A.R., in 20 ml. of freshly deionized water to make a saturated solution. This solution must be made up fresh and should be colorless. Glacial acetic acid, A.R.; methylene chloride, A.R.; carbon dioxide, research grade; and standardized sodium thiosulfate solution, 0.1 *N*.

**Procedure**—*Sampling Procedure for Stability Assays*—Accurately weigh a sample equivalent to 250–275 mg. of benzoyl peroxide into a screw-top glass vial. Record the exact weight and place an identifying mark on the vial. Repeat this procedure for as many analyses as required for the entire study. Place the vials in a controlled temperature oven at the desired test temperature. Two vials should be removed from the oven at predetermined intervals for (duplicate) stability assays.

For initial assays, weigh the sample into a tared 500-ml. iodine flask. Pipet in 20.0 ml. of glacial acetic acid and proceed as directed under *Sample Preparation and Analysis*, beginning with "Add 10 ml. of methylene chloride . . . ."

*Sample Preparation and Analysis*—To the sample vial, add 5.0 ml. (pipeted) glacial acetic acid and carefully wash the entire contents into a 500-ml. iodine flask. Repeat three additional times (total, 20.0 ml. acetic acid), taking care that no residue remains in vial. Using a graduated cylinder, add 10 ml. methylene chloride and swirl to dissolve sample (lotions and creams may leave a turbid solution). Purge the solution for exactly 2 min. with carbon dioxide. Immediately pipet in 5.0 ml. of saturated sodium iodide solution and stopper. Swirl and stand solution in the dark for 30 min. After 28 min. have elapsed, purge 200 ml. of deionized water for 2 min. with carbon dioxide. Add 50 ml. of this deaerated water to the solution. Swirl and titrate to a colorless end point with 0.1 *N* sodium thiosulfate solution. Near the end point, deaerated starch T.S. may be used as an indicator.

$$\text{benzoyl peroxide } \%(\text{w/w}) = \frac{(\text{ml. thiosulfate}) \times (N \text{ thiosulfate}) \times 0.1211 \times 100}{\text{Gm. of sample}}$$

where 0.1211 = meq. wt.

### Polarographic Assay

**Apparatus**—Polarograph (recording polarograph preferred) equipped with an H cell, using a dropping mercury electrode (2–7 sec. drop time) as recording electrode and a saturated calomel electrode as reference electrode.

**Reagents**—Benzene, A.R.; methanol, A.R.; ammonia, A.R. concentrated; methyl red, 0.2% w/v in methanol.

**Procedure**—Accurately weigh a sample equivalent to 10 mg. of benzoyl peroxide into a 250-ml. volumetric flask. Add 50 ml. of benzene, shake for 15 min. on a shaker, add 100 ml. of methanol, and swirl. Add, in order, 5 ml. of ammonia and 2.5 ml.

of 0.2% methyl red solution. Swirl, dilute to volume with methanol, and mix thoroughly. Transfer a portion to the sample side of the H cell. Bubble nitrogen or hydrogen through the solution for 10 min. Polarograph the solution from +0.45 v. to −0.2 v. . . $E_{1/2}$ = +0.27 v. *versus* S.C.E.

Calculate the diffusion current $i_d$ in the conventional manner [limiting current ($i_l$) − residual current ($i_r$)].

Prepare a standard by dissolving an accurately weighed sample of approximately 100 mg. of benzoyl peroxide in benzene and diluting to 100 ml. Transfer a 10-ml. aliquot to a 250-ml. volumetric flask, add 40 ml. of benzene, and continue as directed above, beginning with "Add 100 ml. of methanol. . ."

$$\text{benzoyl peroxide } \%(w/w) = \frac{\frac{i_d \text{ sample}}{i_d \text{ std.}} \times \text{concn. BP std. (mg./ml.)} \times 25}{\text{Gm. of sample}}$$

where BP = benzoyl peroxide.

## RESULTS AND DISCUSSION

A comparison of the titrimetric and spectrophotometric methods was performed to determine the accuracy and precision of the two procedures. Analyses were performed on a reagent grade benzoyl peroxide and a nonflammable pharmaceutical grade (35% active). Known concentrations were incorporated in an oil-in-water lotion and analyses repeated. Table I indicates the results of duplicate assays and demonstrates that all three methods are interchangeable for pure or freshly compounded mixtures. The polarographic procedure was eliminated as an analysis of reagent grade benzoyl peroxide. The diffusion current constant obtained, however, was used for the calculation of peroxide content of the other preparations.

TABLE I—COMPARISON OF RESULTS BY TITRIMETRIC, SPECTROPHOTOMETRIC, AND POLAROGRAPHIC METHODS ON PURE BENZOYL PEROXIDE POWDER AND FRESHLY PREPARED MIXTURES

| | Method | | |
|---|---|---|---|
| | Titrimetric, % | Spectrophotometric, % | Polarographic, % |
| Benzoyl peroxide, 99% | 98.91 | 98.94 | . . . |
| Benzoyl peroxide, 35% | 35.00 | 35.01 | 35.00 |
| Oil-in-water lotion (= 5.29% benzoyl peroxide) | 5.25 | 5.33 | 5.28 |

Based on these findings, stability studies involving the three methods were instituted on commercial preparations containing 5 and 10% benzoyl peroxide. Two of these are lotions manufactured in the United States; the third is a cream produced in Canada.

One of these commercial formulations differs from the others in two respects. First, the benzoyl peroxide is packaged separately as a nonflammable powder containing 35% peroxide which is added by the pharmacist at the time the prescription is dispensed. Second, the pharmacist is directed to place an expiration date on the product at the time of compounding. The other formulations bear no expiration date, implying indefinite stability.

Additional tests were performed on the separately packaged powder to determine the stability of the peroxide in the mixture. The powder was tested at 49° C. and 60° C., while the finished formulations were tested at 46° C. The temperature of 46° C. (115° F.) was chosen since it is not uncommon for this temperature to be encountered in transit and in storage areas during the summer months, and in warmer climates for even longer periods. The powder was tested at higher temperatures because of the increased stability of benzoyl peroxide in the dry form. The results for the dry material, as determined by spectrophotometric analysis and polarographic assay, are indicated in Table II.

TABLE II—STABILITY ANALYSIS OF BENZOYL PEROXIDE POWDER

| Temp. | Benzoyl Peroxide Content— Initial, % | 8 Wk., % | 27 Wk., % |
|---|---|---|---|
| 49°C. (120° F.) | 34.2 | . . . | 33.6 |
| 60°C. (140° F.) | 34.4 | 33.1 | . . . |

This benzoyl peroxide appears to be quite stable at 60° C. after 8 weeks and at 49° C. even after 27 weeks The stability may be attributable to the filler employed (dicalcium phosphate), but no attempt has been made to ascertain the reasons for this since they are outside the scope of this study.

In order to test all three medications under the same condition, the 35% benzoyl peroxide mixture was added to its lotion base (lotion 1) as directed. Figures 1–3 summarize the results of 6.5 weeks of accelerated testing at 46 °C.

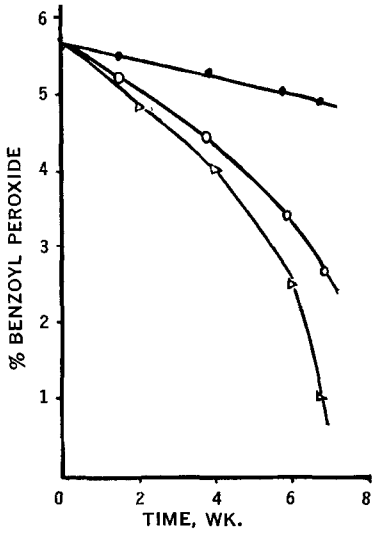


*Fig. 1—Lotion 1. Key: ●, titrimetric; ○, spectrophotometric; △, polarographic.*

The results of the three methods indicate some rather interesting discrepancies. According to the titrimetric procedure, both lotion formulations contained more than 90% of their original peroxide concentrations at the end of the 6.5-week testing period.

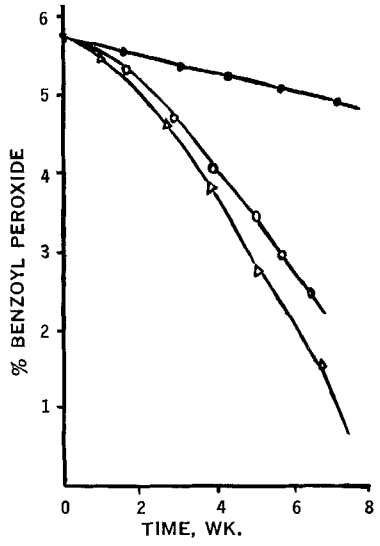


*Fig. 2—Lotion 2. Key: ●, titrimetric; O, spectrophotometric; △, polarographic.*

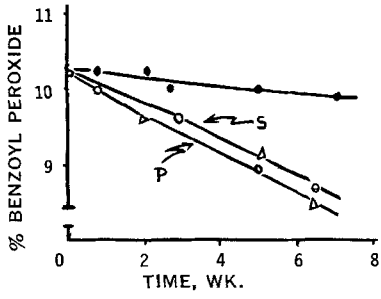


*Fig. 3—Cream. Key: ●, titrimetric; O, spectrophotometric* (S); △, *polarographic* (P).

Similarly, the cream formulation yielded nearly 97% of the benzoyl peroxide initially found. The spectrophotometric method, however, indicates a loss of more than 50% potency for both lotions, while the cream showed a loss of 17%. The polarographic method indicated losses, similar to those found spectrophotometrically, to approximately 70% of original potency. Below this point the divergence becomes significant and increases to yield a nearly 70% loss in one lotion and 78% in the other after 6.5 weeks' storage at 46°C. It is also noteworthy that a secondary wave appeared with $E_{1/2}$ values in the region of 0 v. *versus* S.C.E. after an apparent peroxide loss of 30%. This wave(s) exhibited sharp fluctuations in recorded microamperages. The intensity of the fluctuations and size of this wave increased with longer storage and decreasing benzoyl peroxide concentration. Continued investigation into the nature of the waves(s) and isolation of the component(s) causing this phenomenon is under way at the time of this writing.

From the available data, it seems that the titration procedure apparently does not differentiate some of the degradation products associated with benzoyl peroxide from the parent compound. The polarographic procedure appears to be the most accurate due to its specificity, particularly where significant breakdown has occurred. These results are in line with the finding of Ricciuti *et al.* (8) as previously noted. The spectrophotometric procedure indicates a significant amount of decomposition under prolonged storage conditions, but does not exhibit the full extent of this deterioration as exposed by the polarographic technique, the disparity becoming significant only below the 70% potency level. Values below this level are of little significance for pharmaceutical purposes since the usually accepted minimum limit of potency is 90% of the specified concentration. The method is simple, straightforward, and of sufficient accuracy above 70% potency to be extremely useful for pharmaceutical control and stability testing.

## SUMMARY

Several commercially available benzoyl peroxide pharmaceuticals were subjected to quantitative analysis by a conventional titration method, a spectrophotometric procedure, and a polarographic assay. Although the titration and spectrophotometric methods compared favorably with each other for analysis of the pure benzoyl peroxide substance, the titration procedure proved inadequate for accurately determining the stability of benzoyl peroxide formulations, as confirmed by polarographic investigation. It is ascertained that a dry combination of benzoyl peroxide (35%) and dicalcium phosphate (65%) forms a stable mixture with long storage life. Both the polarographic and spectrophotometric procedures indicate that benzoyl peroxide dispersed in a lotion or cream vehicle has a limited shelf-life and should bear an expiration date. Because of its failure to discriminate between benzoyl peroxide and some of its associated decomposition products, the titrimetric technique cannot be applied with reliability to stability studies of these products.

The preferred method of analysis for adequate control and stability evaluation of pharmaceutical lotions and creams containing benzoyl peroxide (simulating elevated temperature conditions as commonly encountered in pharmaceutical transit and storage), where potency levels must be maintained at 90% or more of the label claim, is a simple spectrophotometric procedure. This method has been demonstrated to be accurate at levels of 70% or more of the original concentration.

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