actual measurement is difficult. Scheme II illustrates such a calculation for methyltestosterone starting from the 17-androstyl group.

Table III summarizes similar calculations of the free energy of transfer of the compounds studied along with the measured partition coefficients taken from the data shown in Fig. 7.

Agreement (within a factor of 2) was obtained between the calculated partition coefficients and the measured values with the exception of methyltestosterone acetate. The measured value for methyltestosterone added to the acetyl group contribution determined from the data for 1-methylcyclopentanol and 1-methylcyclopentyl acetate gave a value of ~5000 for the partition coefficient, which is in much better agreement with the experimental value.

This study shows that in cases where experimental values are difficult to obtain, estimates based on the group contribution approach are reasonably accurate.

If one takes the measured solubility in isooctane and divides by the partition coefficient as an approximation to the solubility in water, reasonable agreement with the measured solubility values is obtained. Thus, in the special case of extremely low solubility in water, estimating the partition coefficient by the group contribution approach and combining it with the known solubility in an organic solvent provide a good estimate of the aqueous solubility. In such cases, this approach may be the only feasible method of obtaining an accurate solubility estimate.

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Determination of Phenylmercuric Nitrate by Potentiometric Titration

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Abstract □ A procedure was developed for measuring small amounts of phenylmercuric nitrate in aqueous solutions. The method depends on the formation of insoluble phenylmercuric iodide upon titration of phenylmercuric nitrate with potassium iodide. The end-point can be detected using an iodide-sensitive electrode. The method is able to measure down to 0.000125% aqueous solution of phenylmercuric nitrate with a 1% accuracy. Procedural details and descriptions of excipient effects on the assay are presented. Naphazoline hydrochloride, phenylephrine hydrochloride, fluorescein sodium, and antipyrine interfered with the method, while the common buffer systems, polyvinyl alcohol, sodium thiosulfate, edetate sodium, and chloramphenicol had no effect.

Keyphrases □ Phenylmercuric nitrate—analysis, potentiometric titration, dilute aqueous solutions, excipients □ Ophthalmic dosage forms—phenylmercuric nitrate, potentiometric titration analysis, dilute aqueous solutions, excipients □ Potentiometric titration—analysis, phenylmercuric nitrate, dilute aqueous solutions

Phenylmercuric nitrate is frequently used as a preservative for ophthalmic and other preparations. To carry out routine determinations of small amounts of phenylmercuric nitrate in aqueous solution, a simple, rapid, and sensitive assay is necessary. Published methods for determining phenylmercuric nitrate have been numerous, but many of these are tedious or lack sensitivity.

BACKGROUND

Microbiological methods (1) are time consuming and are only semiquantitative. Spectrophotometric methods take advantage of the UV absorption at 257 nm by phenylmercuric nitrate (2). Although this assay is rapid, its sensitivity is limited. Many procedures involve conversion of organomercury to mercuric ion, followed by classical thiocyanate titrimetry (3). Again, this method lacks the sensitivity required for very dilute solutions.

The British Pharmacopoeia lists the dithizone extraction method (4), which involves extraction of the mercury from an acidic solution with a solution of dithizone in chloroform. The dithizonate is then measured spectrophotometrically. Because of the extractions involved, this method is tedious for routine determinations.

Ordinary polarographic methods have been used for higher phenylmercuric nitrate concentrations (5); cathode ray polarography of phenylmercuric nitrate has been investigated (6) and is a simple, rapid, and sensitive assay for routine determinations. Furthermore, phenylmercuric nitrate has been detected satisfactorily by atomic absorption spectroscopy (7). This procedure is based on "protodemercuration" of the mercurial compound with hydrochloric acid under various heating conditions, followed by reduction of the resulting mercuric ion to elemental mercury with subsequent detection and quantitation by vapor phase atomic absorption spectroscopy. Since these procedures require instruments that may not be readily available, the technique described in this paper was developed. This relatively rapid, simple, and sensitive assay is based on the precipitation of the phenylmercuric moiety with iodide ion.

Insoluble phenylmercuric iodide, $K_{sp} = 9.7 \times 10^{-16}$ (8), is formed when an aqueous solution containing phenylmercuric ions is titrated with iodide according to Scheme I.

$$C_6H_5—Hg^+ + I^- \rightleftharpoons C_6H_5—HgI$$

Scheme I

In the developed procedure, the end-point for this reaction is detected potentiometrically using an electrode sensitive to iodide ions.

EXPERIMENTAL

Five milliliters of an aqueous phenylmercuric nitrate[1] solution was transferred to a 50-ml beaker equipped with a magnetic stirring bar. The amount of phenylmercuric nitrate in solution ranged from ~0.05 to 2.5

[1] Lot 0900030, British Drug Houses Chemicals Ltd., Poole, England.

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Table I—Comparison of Known and Measured Phenylmercuric Nitrate Concentrations

Known Phenylmercuric Nitrate Concentration, % w/v	Measured Concentration, % of known[a]
0.0100	99.4 ± 0.06
0.00500	98.4 ± 0.00
0.00200	99.5 ± 0.00
0.00100	101.7 ± 1.15
0.000500	100.6 ± 2.06
0.000250	100.0 ± 1.13
0.000125[b]	98.9 ± 0.92

[a] Average of three determinations ± *SD*. [b] The lowest concentration that the assay could detect with confidence.

mg. Twenty milliliters of distilled water was added to make a workable volume, and the solution was acidified with concentrated sulfuric acid[2]. This solution was titrated, using a potentiograph[3], with a solution of known concentration of potassium iodide[4] saturated with iodine[5]. The potassium iodide concentration was approximately equal to the concentration of phenylmercuric nitrate being assayed. The solution was stirred continuously *via* a magnetic stirrer.

A platinum electrode[6] was used to detect the iodide concentration, and a calomel electrode[7] equipped with a potassium nitrate[8] salt bridge was used as the reference electrode. The end-point of each titration was read directly from the recording chart of the potentiograph.

The iodine-saturated potassium iodide solution was standardized by titrating a known amount of analytical standard phenylmercuric acetate[9] with the potassium iodide solution.

RESULTS AND DISCUSSION

The potentiometric method is based on the ability of the electrode to detect the sudden increase of iodide ions once the phenylmercuric cation in the solution has precipitated as phenylmercuric iodide. Platinum in contact with iodine is sensitive to iodide (9) and was used as the indicating electrode. The iodine necessary for detecting the iodide ions was present in the potassium iodide solution. In a solution that contains iodide ion and molecular iodine, a reaction between the two species occurs as in Scheme II.

$$I_2 + I^- \rightleftharpoons I_3^-$$

Scheme II

Then, according to the law of mass action:

$$K = \frac{[I_3^-]}{[I_2][I^-]} \qquad \text{(Eq. 1)}$$

At 18°, K is equal to 8.70×10^2 (9). Therefore, in fact, the phenylmercuric nitrate is being titrated by the I_3^- species, which consequently generates the I^- species. Kolthoff and Furman (9) suggested that, for the titration of mercuric ions with iodide, I_2 be introduced as an alcohol solution into the solution being titrated. However, when this was done, end-point reproducibility became a problem. This result was probably due to competition for the iodide ion by the phenylmercuric cation and the molecular iodine or to the formation of small amounts of iodide from the iodine (9). When the iodine is in the potassium iodide solution, the competition is prevented and the added iodide is accounted for during the standardization.

As an alternative to platinum in contact with iodine to detect the iodide ions, one may use an iodide-specific electrode[10]. Several determinations made with such an electrode gave results similar to those obtained with the platinum electrode.

Figure 1 shows a plot of potential *versus* titrant volume. Equilibrium apparently was established rapidly between the species involved in the titration. Therefore, no drift was observed, and the end-point was sharp. The sulfuric acid, which was added to the phenylmercuric nitrate solution before the titration began, was crucial to the sharpness of the end-point. This observation may be similar to that seen in the titration of iodide with silver nitrate (9). When the titration was carried out in acidic solution, the phenylmercuric iodide flocculated from the start of the titration, whereas in a neutral medium the iodide remained in colloidal solution and flocculated just before the equivalence point was reached. Owing to the high degree of dispersion, the adsorbing capacity of the iodide was much greater in the latter case than in an acidic medium. However, too much sulfuric acid caused reproducibility problems. About 5 drops of concentrated sulfuric acid sufficed.

Table II—Effect of Excipients on Assay of 0.002% Phenylmercuric Nitrate Solution

Excipient	Percent, w/v	Percent of Original Concentration[a]
Boric acid	1.00	98.3 ± 1.04
Sodium borate	0.05	98.6 ± 1.26
Acetic acid	0.005	99.7 ± 1.04
Sodium acetate	1.0	99.5 ± 0.87
Dibasic sodium phosphate	1.0	99.8 ± 0.58
Monobasic sodium phosphate	1.0	100.3 ± 0.76
Polyvinyl alcohol	1.4	100.5 ± 0.58
Sodium thiosulfate	0.1	100.4 ± 0.58
Edetate sodium	0.1	99.5 ± 1.15
Chloramphenicol	0.5	89.3 ± 0.29[b]
Naphazoline hydrochloride	0.1	0[b]
Phenylephrine hydrochloride	0.12	0[b]
Fluorescein sodium	0.1	0[b]
Antipyrine	0.1	0[b]

[a] Average of three determinations ± *SD*. [b] Significantly different from original phenylmercuric nitrate concentration.

To check the sensitivity, precision, and accuracy of the analytical procedure followed for phenylmercuric nitrate, measurements were made with known amounts of phenylmercuric nitrate (Table I). The accuracy throughout the concentration range was ~1%. The lowest measurable concentration was 0.000125%. Below this concentration, the end-points were not sharp enough. A t-test performed on these data showed that the

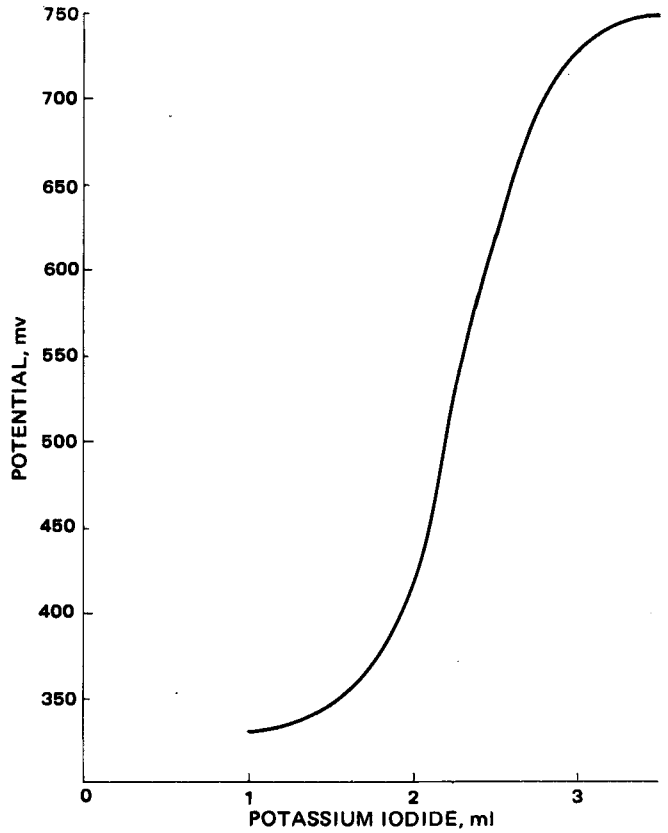

Figure 1—*Potential change for the titration of 0.002% phenylmercuric nitrate with 0.002% potassium iodide.*

[2] ACS approved, J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[3] Model E 336 A, Metrohm, Herisau, Switzerland.
[4] Lot 83176, British Drug Houses Chemicals Ltd., Poole, England.
[5] Lot 710919, laboratory grade, Fisher Scientific Co., Fair Lawn, NJ 07410.
[6] Model EA201, Metrohm, Herisau, Switzerland.
[7] Model EA436, Metrohm, Herisau, Switzerland.
[8] Lot 723124, laboratory grade, Fisher Scientific Co., Fair Lawn, NJ 07410.
[9] Lot 12028, analytical standard, British Drug Houses Chemicals Ltd., Poole, England.
[10] Model 94-53A, Orion Research Inc., Cambridge, MA 02139.

average of the results is not significantly different from 100%, so the method does not appear to have any bias.

Ophthalmic products that contain phenylmercuric nitrate have other substances in the solution such as the active drug, reducing agents, buffer systems, and surfactants. To assess the effectiveness of the assay for phenylmercuric nitrate in ophthalmic products, the effect of these other substances on the assay was determined. Table II shows the approximate concentration of the excipient used to determine its effect on the assay. Concentrations of buffer pairs were such that the solutions were isotonic and at pH 7.

Table II shows four excipients that interfered completely with the phenylmercuric nitrate assay; *i.e.*, no end-point could be obtained. Although they are used in commercial products (10) with phenylmercuric nitrate, the drugs naphazoline hydrochloride and phenylephrine hydrochloride probably cause precipitation of insoluble phenylmercuric chloride even before the titrating begins, as shown in Scheme III.

$$C_6H_5—Hg^+ + Cl^- \rightleftharpoons C_6H_5HgCl$$

Scheme III

The K_{sp} for this reaction is 5.0×10^{-10} (8); therefore, the solubility limit of phenylmercuric chloride is exceeded in the phenylmercuric nitrate solutions containing naphazoline hydrochloride and phenylephrine hydrochloride. This would no doubt also happen in solutions of other halide salts. It was reported that antipyrine complexes with mercury compounds (11). If this complexation occurs with phenylmercuric nitrate, it might prevent the formation of the insoluble phenylmercuric salt, which is crucial to the titration. In contrast to the other systems, the effect of fluorescein sodium on the assay and the significant decrease of phenylmercuric nitrate in the presence of chloramphenicol remain unexplained.

In summary, this method of analysis for phenylmercuric salts should prove useful in applications requiring phenylmercuric nitrate determination in dilute solution.

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Stereospecific Assay and Stereospecific Disposition of Racemic Carprofen in Rats

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Received January 11, 1979, from the **Department of Biochemistry and Drug Metabolism and the* ‡*Department of Toxicology, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication April 18, 1979.

Abstract □ A procedure was developed for the separation and selective quantitative determination of the (*S*)(+)- and (*R*)(−)-enantiomers of the racemic anti-inflammatory drug carprofen as their diastereomeric *l*-(−)-α-methylbenzylamides. These derivatives are obtained in equivalent yields by reacting purified ^{14}C-carprofen from biological specimens with *l*-(−)-α-methylbenzylamine *via* the 1,1′-carbonyldiimidazole intermediate, followed by extraction and differential radiometric quantitation of the TLC-separated diastereomers. In the rat, the (*R*)(−)-carprofen enantiomer was eliminated from blood and secreted in the bile as the ester glucuronide at a rate approximately twice that of the (*S*)-(+)-enantiomer, resulting in the accumulation of the pharmacologically more active (*S*)(+)-enantiomer in the rat blood. Evidence for an additional process favoring the elimination of the (*R*)(−)-enantiomer in the rat was derived from pharmacokinetic data evaluation.

Keyphrases □ Carprofen—racemic mixtures, stereospecific assay, stereospecific metabolism, rats □ Enantiomers—carprofen, stereospecific assay, stereospecific metabolism, rats □ Anti-inflammatory agents—carprofen, racemic mixtures, stereospecific assay, stereospecific metabolism, rats

The anti-inflammatory agent carprofen (1), (D,L)-6-chloro-α-methylcarbazole-2-acetic acid (I), is a racemic compound with a chiral center at the α-carbon. The optically active carprofen enantiomers have been resolved, and their confirmations have been identified by X-ray analysis[1].

The properties of the dextrorotatory (*S*)(+)-enantiomer (II) and the levorotatory (*R*)(−)-enantiomer (III) have been compared in biological *in vivo* and *in vitro* tests (1). In the acute adjuvant arthritis test in the rat, the (*R*)-(−)-enantiomer was less than one-tenth as active (ID_{30}) as the (*S*)(+)-enantiomer. In contrast to the latter, the former was virtually inactive as an inhibitor of platelet aggregation, prostaglandin synthetase, and arachidonic acid-induced diarrhea; it produced no ulcerogenesis nor any other *in vivo* manifestation of toxicity in the rat.

Therefore, a stereoselective disposition of the two carprofen enantiomers could result in a different pharmacokinetic profile of the biologically active enantiomer from that previously determined by nonstereospecific procedures following administration of the racemate (2, 3). Possible stereoselective drug disposition processes include selective biotransformation reactions, tissue uptake,

[1] The identification by X-ray crystallography of the chiral configuration of the carprofen enantiomers was performed by Dr. J. F. Blout of the Roche Department of Physical Chemistry.

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