Determination of Nitrofurantoin in Urine by Reduction at Rotating Platinum Electrode

WILLIAM D. MASON ˣ and BEVERLY SANDMANN

Abstract □ The application of the reduction of nitrofurantoin at the rotating platinum electrode to the determination of the drug in the urine of clinical patients was evaluated and compared to a commonly used colorimetric method. The new electrochemical method was found to be faster and more efficient with precision and accuracy comparable to those of the colorimetric method.

Keyphrases □ Nitrofurantoin—electrochemical analysis, compared to colorimetric analysis, urine □ Electrochemical analysis—nitrofurantoin, urine, compared to colorimetric analysis □ Antibacterial agents—nitrofurantoin, electrochemical analysis, urine

The use of nitrofurantoin, 1-[(5-nitrofurfurylidene)amino]hydantoin, in the treatment of urinary tract infections is widely accepted (1). Due to the site of action, determination of the concentration of nitrofurantoin in urine is important for bioavailability studies and patient monitoring (2). The commonly used method for the determination of nitrofurantoin in urine is a colorimetric procedure (3–5), based on extraction of the drug into nitromethane followed by the formation of a nitrofurantoin–hyamine complex which absorbs at 400 nm.

A polarographic method was reported for the determination in urine, based on measuring the peak current of the differential polarogram resulting from reduction of the nitro group (6). A standard addition technique is employed to correct for possible differences in the diffusion constant of the drug in various urine samples.

Although the reported methods are sufficiently specific and sensitive, they involve cumbersome manipulative steps or the use of equipment frequently not available in laboratories doing clinical assays (*i.e.*, a differential or differential pulse polarograph). The rotating platinum electrode, because of the higher limiting currents possible with combined convection–diffusion-controlled mass transport, was considered a possible alternative to the common mercury electrodes.

The rotating platinum electrode may be used as the working electrode in a simple direct current procedure with high sensitivity. Thus, an investigation of the reduction of nitrofurantoin at the rotating platinum electrode, with the objective of developing a simple and rapid method for the determination of this drug in urine, was undertaken. The developed method is presented and compared to the commonly employed colorimetric method (3).

EXPERIMENTAL

Apparatus—The three-electrode cell with the rotating platinum electrode is shown in Fig. 1. The rotating platinum electrode is turned by a synchronous rotator[1] at the rate of 1800 rpm. The working and counter electrodes are made of platinum wire sealed in glass, and the reference electrode is a saturated calomel electrode[2]. The cell is designed to require 15 ml of solution for analysis and to permit rapid conversion from one sample to the next.

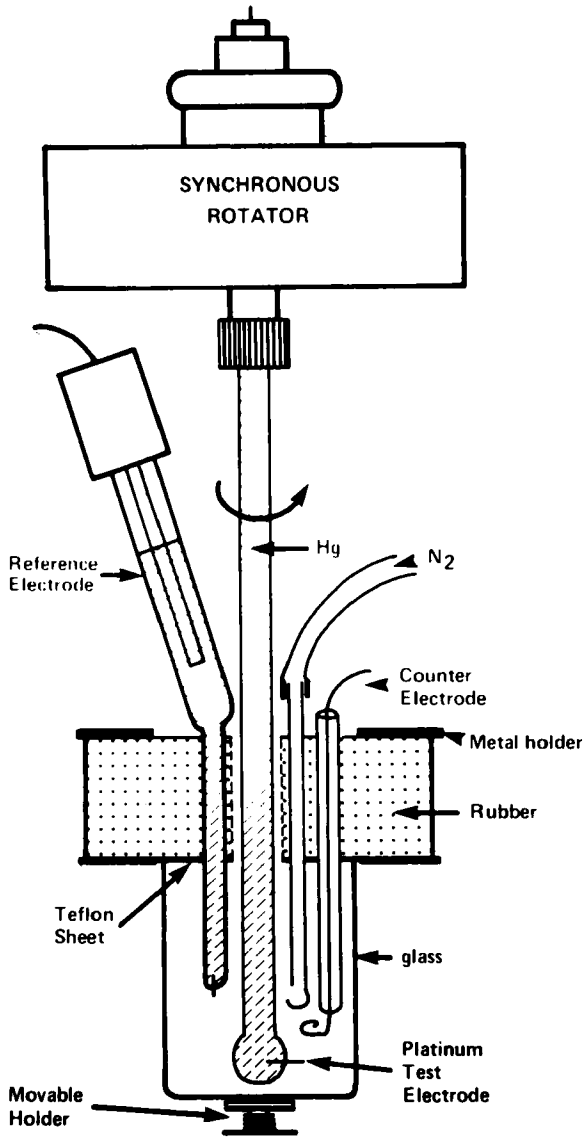

Figure 1—*Three-electrode cell for rotating platinum electrode.*

A dc polarography system[3] is used to record the current–voltage curves. All electrical leads are shielded, and the shields are connected to ground.

Chemicals—All chemicals were of the highest quality commercially available. Standard solutions of nitrofurantoin were prepared in dimethylformamide or acetone from a pure powder[4].

Procedures—Current–voltage curves were determined by placing about 15 ml of the test solution in the electrolysis cell, deaerating with nitrogen for 4 min with the voltage at 0 v *versus* the satu-

[1] S-76485, Sargent Welch Scientific Co.

[2] Coleman No. 3-152.

[3] Heath EU-401.

[4] Norwich Pharmacal Co., Norwich, N.Y.

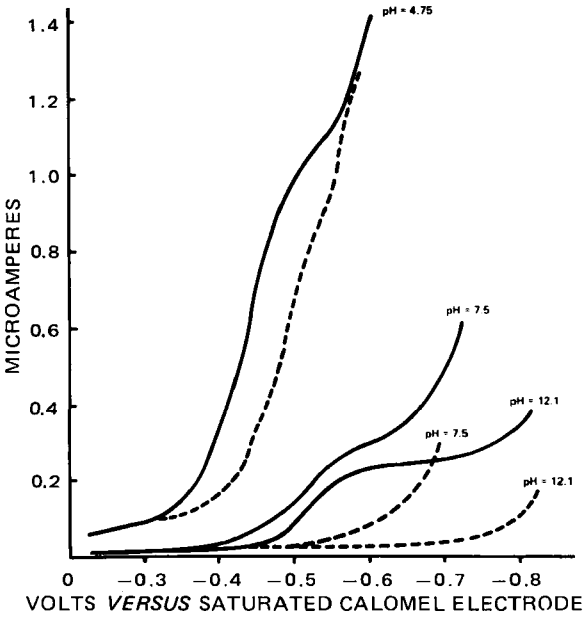

Figure 2—*Nitrofurantoin current voltage curve for 4-μg/ml solutions in various buffers. Key: —, nitrofurantoin; and - - -, buffer only.*

rated calomel electrode. Then the voltage was scanned cathodically at 5 mv/sec with the rotating platinum electrode turning at 1800 rpm. During the scan, the nitrogen atmosphere was maintained above the solution. Holding the electrode potential at 0 v *versus* the saturated calomel electrode during the deaeration period was necessary to condition the electrode.

Several buffer solutions were evaluated to determine their effects on the reduction wave of nitrofurantoin and the background current at the rotating platinum electrode. The buffers studied were 0.1 *M* H_2SO_4, 0.2 *M* HCl, 0.5 *M* sodium acetate (pH 4.75), 0.2 *M* sodium phosphate (pH 6.5 and 7.5), 0.5 *M* sodium phosphate (pH 6.5 and 7.5), 0.2 *M* sodium borate (pH 9.0 and 12.1), 0.5 *M* sodium borate (pH 9.0 and 12.1), and 0.25 *M* sodium hydroxide.

Urine samples were collected from 20 different hospitalized patients who were not taking nitrofurantoin or other drugs containing nitro groups. However, each patient was taking one or more of the following drugs: aspirin, acetaminophen, codeine, diazepam, chlordiazepoxide, phenytoin, phenobarbital, quinidine, or tolbutamide. Five milliliters of each urine sample was diluted to 50 ml in each buffer, and current–voltage scans were run. Then nitrofurantoin (to give 40 μg/ml) was added to each urine sample, and the current–voltage scans were repeated. These spiked urine samples also were assayed by the colorimetric method (3).

The accuracy and precision of the rotating platinum electrode method were compared to the colorimetric method (3) by analysis of urine samples from five patients taking nitrofurantoin for urinary infections. The rotating platinum electrode method consisted of pipetting 5.0 ml of the urine into a 50-ml volumetric flask and diluting to volume with 0.50 *M* borate buffer (pH 12.1). Then the current–voltage curve was determined, and the concentration was calculated from a calibration curve of limiting current *versus* nitrofurantoin concentration.

RESULTS AND DISCUSSION

The current–voltage curve for reduction of nitrofurantoin at the rotating platinum electrode is greatly dependent on the pH and molarity of the buffer system employed. In strong acid media, the nitrofurantoin wave and background current were not separated and thus were not useful for analysis. Figure 2 shows some typical current–voltage curves at three pH values; the more alkaline pH was required for a suitable current–voltage curve.

It also was noted that the higher molarity buffers (*i.e.*, 0.5 *versus* 0.2 *M*) gave sharper current–voltage curves. The current–voltage scans run in 0.25 *M* sodium hydroxide gave a limiting current, which demonstrated the lack of stability of nitrofurantoin in this medium. The limiting current decreased 17% in 10 min. In the 0.5

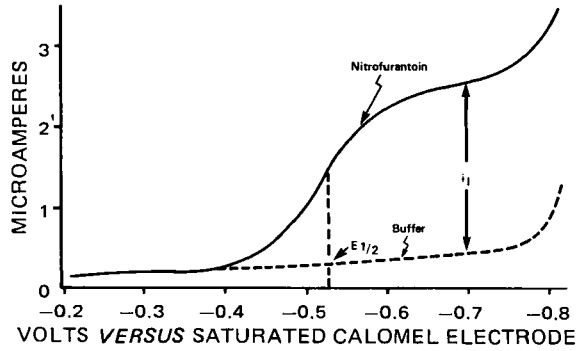

Figure 3—*Reduction of nitrofurantoin (4 μg/ml) in 0.50* M *borate buffer, pH 12.1.*

M sodium borate buffer (pH 12.1), the limiting current decreased 4.0% in 60 min. Thus, the borate buffer can be used in the analysis if test solutions are assayed within a few minutes of preparation.

Figure 3 shows a typical current–voltage curve for nitrofurantoin reduction at the rotating platinum electrode in 0.5 *M* sodium borate buffer. The half-wave potential was −0.53 v *versus* the saturated calomel electrode, and $E_{3/4}$–$E_{1/4}$ was 75 mv. The limiting current varied in a linear manner with the concentration of nitrofurantoin. A typical calibration curve between 0.50 and 15 μg/ml is expressed by:

$$i_l = \left(0.39 \frac{\mu\text{amp ml}}{\mu\text{g}}\right) C + 0.39\ \mu\text{amp} \qquad \text{(Eq. 1)}$$

with a regression coefficient of 0.98 when the limiting current was determined in triplicate at 0.5, 1, 2, 4, 8, 10, 14, and 15 μg/ml. Calibration plots determined at various times over a month did not differ more than 5.0% in either slope or intercept.

The current–voltage curves on the urine samples from patients not taking drugs with nitro groups showed no reduction waves and thus no potential interferences. The limiting current for nitrofurantoin (4.0 μg/ml) in 90% sodium borate buffer and 10% urine from the 20 patients not taking drugs with nitro groups ranged from 1.66 to 1.87 μamp. This study indicates the small potential error introduced by the urine from various sources and that interference due to commonly used drugs or their metabolites is unlikely.

The possible interferences of various antibiotics, particularly chloramphenicol, were not evaluated, because no patients were encountered who were taking such combinations. Indeed, clinicians indicate that such therapy seldom would be employed. The rotating platinum electrode method involves the reduction of the nitro group, so drugs (*i.e.*, nitroimidazoles and chloramphenicol) or drug metabolites containing nitro groups and appearing in high concentration in the urine would interfere.

Table I shows the comparative values and precision of the rotating platinum electrode method and the colorimetric method (3) for samples from five patients taking nitrofurantoin. The colorimetric method is more precise, but the mean values obtained are quite close.

SUMMARY

In summary, a relatively simple and fast method for the determination of nitrofurantoin in urine is presented. The method allows a triplicate analysis in 35 min, with only a slight loss in the

Table I—Comparative Analysis of Urine from Patients Taking Nitrofurantoin

Patient	Mean ± *SD*[a], μg/ml: Rotating Platinum Electrode Method	Mean ± *SD*[a], μg/ml: Colorimetric[b] Method
1	20.0 ± 0.5	21.2 ± 0.2
2	43.0 ± 1.0	44.0 ± 0.5
3	3.5 ± 0.5	3.8 ± 0.1
4	24.0 ± 0.8	23.0 ± 0.2
5	71.0 ± 1.5	69.0 ± 0.8

[a] Three assays on each sample. [b] Reference 3.

precision obtained with the longer colorimetric method. The new method does not require extraction steps or the addition of reagents, both of which take considerable time.

The method described is similiar to the method of Jones *et al.* (6) in that it is based on the electrochemical reduction of the nitro functional group in nitrofurantoin. The rotating platinum electrode method has the advantage over a previously used electrochemical method of not requiring a differential pulse polarograph or differential polarograph; it requires only a simple direct current polarograph, because of the relatively high sensitivity possible with a rotating electrode.

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[x] To whom inquiries should be directed.

Simple Transformation Method for Predicting Plasma Drug Profiles from Dissolution Rates

D. P. VAUGHAN [x] and R. H. LEACH [‡]

Abstract □ A transformation factor is described which related *in vitro* drug dissolution from a preparation to the corresponding *in vivo* plasma drug concentrations. This factor, derived from the dissolution profile and the corresponding *in vivo* plasma concentration of a single formulation, was used to predict plasma concentration profiles of similar formulations simply from dissolution data.

Keyphrases □ Plasma drug profiles—predicted from dissolution rates, transformation factor described □ Dissolution rates—transformation factor described for prediction of plasma drug profiles

Frequently, the *in vivo* dissolution rate of an oral dosage form controls the rate at which a drug appears in plasma, and similar formulations of the same drug can have different therapeutic equivalences. Since drug absorption studies in humans are expensive and time consuming, *in vitro* dissolution data are frequently correlated with the biological availability of a drug from different preparations. The times to achieve 50% drug dissolution (t_{50}) usually are correlated with either maximal plasma drug concentrations or the areas under the plasma concentration–time curves (1).

Although these latter methods are useful, they are not designed to predict the plasma time course of a drug following administration of different preparations. One method of predicting plasma drug concentrations involves a detailed pharmacokinetic study of drug distribution and elimination after an oral aqueous dose and the derivation of an explicit function to describe *in vitro* drug dissolution from various preparations (2).

Alternatively, a curve follower or variable diode function generator can be used to input *in vitro* dissolution data directly into a pharmacokinetic model programmed on an analog computer (3).

To avoid detailed pharmacokinetic studies in humans, a simple transformation method was investigated for predicting plasma drug concentrations from *in vitro* dissolution data.

THEORY

Two independent functions of time, $f_1(t)$ and $f_2(t)$, can be related to each other at some specific value of time by an arbitrary transformation factor, $m(t)$, so that at time t:

$$f_1(t)m(t) = f_2(t) \qquad \text{(Eq. 1)}$$

Relating the two functions (Eq. 1) in this way does not necessarily imply a specific relationship between them. Similarly, *in vitro* dissolution characteristics of an oral drug preparation can be related to the corresponding *in vivo* plasma drug concentrations.

Without defining the distribution and elimination processes in the body, the plasma concentration of a drug obtained with some drug input process into the body can be related to the drug input by an operator, $m(t)$, such that:

$$\text{drug input}(t) = m(t)\ [\text{plasma drug concentration}(t)] \qquad \text{(Eq. 2)}$$

In Eq. 2, m is a collection of numbers which, together with the drug input, uniquely determines the plasma drug concentration for all $t \geq 0$. When considering plasma drug concentrations obtained after oral administration of a dosage formulation, Eq. 2 becomes:

$$\text{amount of drug released } \textit{in vivo} \text{ from formulation}(t) = m_i(t)\ [\text{plasma drug concentration}(t)] \qquad \text{(Eq. 3)}$$

where m_i is a collection of numbers which, together with the amount of drug released from the preparation at time t, uniquely determines the plasma drug concentration at time t for all $t \geq 0$.

Similarly, *in vitro* drug dissolution from an oral formulation can be related to the corresponding *in vivo* drug dissolution by an operator, $m_j(t)$:

$$\text{amount of drug dissolved in } \textit{in vitro} \text{ dissolution test from dosage formulation}(t), \textit{i.e.}, \text{dis}_j(t) = m_j(t) \times \text{amount of drug released } \textit{in vivo} \text{ from formulation}(t) \qquad \text{(Eq. 4)}$$