New method for spectrophotometric determination of quinones and barbituric acid through their reaction. A kinetic study

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Abstract

A new and sensitive spectrophotometric method is described for the determination of p-benzoquinone, p-chloranil and 1,4-naphthoquinone. The method is based on the reaction between quinones and barbituric acid, by which a color is developed with maximum absorption between 485 and 555 nm in 50% methyl alcohol–water mixture. The absorption of the product obeys Beer's law within the concentration range 0.025–0.05 mM of orginal quinone. The kinetics of the reaction between p-benzoquinone and barbituric acid was studied in a range of methyl alcohol–water mixtures. The reaction follows overall second order kinetics, first order in each of the reactants. The rate increases with increasing dielectric constant. The method was applied for determination of barbituric acid with p-benzoquinone in the concentration range of 0.025–0.345 mM. Other barbiturates do not interfere.

Keywords: Barbituric acid; Quinones

1. Introduction.

Quinones are compounds of wide occurrence in nature. Their importance in biochemistry as bacteriostatic [1] agents, their fungicidal action [2], and their inhibitory influence on certain enzymes, such as carboxylase and urease [1], makes it desirable to have an accurate method for the analytical determination of these quinones.

Various methods commonly applied for quinone determination have applied in literature and comprise titrimetric [3,4], potentiometric [5,6], differential pulse polarographic [7] and spectrophotometric [8–13] methods.

The presently reported work aimed to develop a simple and sensitive spectrophotometric method for kinetic determination of quinones via their interaction with barbituric acid.

2. Experimental

2.1. Materials and reagents

1,4-naphthoquinone and p-chloranil (BDH) were used without further purification. p-Benzoinone (BDH) was purified by sublimation [14]. Alcoholic solutions of barbituric acid (Fisher, highest purity) was freshly prepared for each analysis. All other chemicals used were of reagent grade.
Table 1
Statistical analysis of the calibration graphs for the determination of some quinones by reaction with barbituric acid in 50% methyl alcohol–water mixture

<table>
<thead>
<tr>
<th>Quinone</th>
<th>( \lambda_{\text{max}} /\text{nm} )</th>
<th>( E_{\text{per}} )</th>
<th>Concentration range /mM</th>
<th>Slope (b)</th>
<th>Intercept (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Benzoquinone</td>
<td>500</td>
<td>60</td>
<td>0.025–0.5</td>
<td>0.0039</td>
<td>0.041</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>485</td>
<td>140</td>
<td>0.025–0.5</td>
<td>0.0127</td>
<td>0.072</td>
</tr>
<tr>
<td>( p )-Chloranil</td>
<td>555</td>
<td>50</td>
<td>0.025–0.5</td>
<td>0.0043</td>
<td>0.017</td>
</tr>
</tbody>
</table>

2.2. Apparatus

Visible absorption spectra were recorded on a UNICAM SP 1800 recording spectrometer. An SP 6-200 PYE-UNICAM spectrophotometer was used for the kinetic spectrophotometry.

2.3. Analytical procedure

A calibration graph for each of the quinones (\( p \)-benzoquinone, 1,4-naphthoquinone and \( p \)-chloranil) was obtained by transferring 0.1–2.0 mL aliquots of \( (2.5 \times 10^{-3} \) M) solutions of them in methyl alcohol to test tubes, followed by addition of 1.0 mL of \( (0.05 \) M) methanolic solution of barbituric acid and an appropriate volume of distilled water to attain 50% v/v mixture. The mixtures were heated at 30°C in a water bath for 5 and 15 min for \( p \)-benzoquinone and \( p \)-chloranil respectively, and at 50°C for 1 h. in the case of 1,4-naphthoquinone. The mixtures were then cooled to room temperature, diluted to 10 mL with 50% methyl alcohol–water mixture and the absorbance of the resulting pink color measured at the wavelength of maximum absorption of each quinone derivative. Beer’s law was found to hold over the concentration range investigated of 0.025–0.5 mM of original quinone. Table 1 shows a linear regression analysis of the data obtained from calibration graphs of the quinones used from the relation \( A = a + bc \), where \( A \) is the absorbance at the relevant peak in a 1.0 cm quartz cell, \( a \) and \( b \) are the intercept and the slope of the calibration graph, respectively, and \( c \) is the concentration of the quinone in mM in the final solution. No interference is caused by the presence of 10 mM formaldehyde, 10 mM acetone, 2.8 mM benzaldehyde, 2.46 mM benzoic acid, 2.4 mM 9,10-anthraquinone and 1.93 mM phenanthrene-quinone.

2.4. Kinetic procedure

The rate of reaction of \( p \)-benzoquinone with barbituric acid was followed by monitoring the increase in absorption of the product at its wavelength of maximum absorption, with time.

Freshly prepared samples of \( p \)-benzoquinones \( 5 \times 10^{-3} \) M and barbituric acid \( (0.05 \) M) in methyl alcohol were allowed to equilibrate to 30°C for 15 min in a thermostat. Mixing was carried out by withdrawing 0.5–2 mL, aliquots of the \( p \)-benzoquinone solution to 10 mL flasks containing 1.0 mL of \( (0.05 \) M) barbituric acid solution, diluted with the appropriate volume of.
Table 2

Kinetic order determination for the reaction between \( p \)-benzoquinone and barbituric acid in 50% methyl alcohol–water mixture at 30°C

<table>
<thead>
<tr>
<th>(10^4 \times [\text{quinone}] / \text{M} )</th>
<th>(10^4 \times [\text{barbituric acid}] / \text{M} )</th>
<th>(10^7 \times \text{(initial rate)} / (\text{mol L}^{-1} \text{s}^{-1}))</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>50.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>50.0</td>
<td>20.8</td>
<td>1</td>
</tr>
<tr>
<td>7.5</td>
<td>50.0</td>
<td>30.2</td>
<td>1</td>
</tr>
<tr>
<td>10.0</td>
<td>50.0</td>
<td>39.8</td>
<td>1</td>
</tr>
<tr>
<td>50.0</td>
<td>2.5</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>5.0</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>7.5</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>10.0</td>
<td>25.1</td>
<td></td>
</tr>
</tbody>
</table>

methyl alcohol–water mixture to attain 50% v/v mixture, and zero time was recorded upon immediate shaking of the flask. A portion of the reaction mixture was transferred to a quartz cuvette placed in the thermostatic (30°C) cell compartment. A blank solution was similarly prepared without quinone and the absorbance readings were also recorded and followed as a function of time. Another set of runs was carried out in a similar manner using \((5 \times 10^{-3} \text{ M})\) barbituric acid and \((0.05 \text{ M})\) benzoquinone. The changes in concentration were calculated from the molar absorption coefficient of the product.

3. Results and discussion

The visible spectra of the colored products of the tested quinones with barbituric acid in a 50% methyl alcohol–water mixture were determined (Fig. 1) and the wavelength of the absorption peaks for each product was noted for use in the analytical and kinetic determinations.

3.1. Order of the reaction

For the reaction of \( p \)-benzoquinone with barbituric acid, the order of reaction was determined by the differential method [15]. The logarithmic relationships between initial rates and initial concentration of the reactants gave straight lines with slopes indistinguishable from unity, as shown in Table 2 and Fig. 2. The reaction is thus first order in each of the reactants, and will follow overall second order kinetics.

Table 3

Second order rate constant \( (k_2) \) for the reaction of \( p \)-benzoquinone \((5 \times 10^{-4} \text{ M})\) with \((5 \times 10^{-4} \text{ M})\) barbituric acid in methyl alcohol–water mixtures at 30°C

<table>
<thead>
<tr>
<th>Methyl-alcohol / wt.%</th>
<th>Dielectric constant ( (D) )</th>
<th>( k_2 / (\text{L mol}^{-1} \text{s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>34.6</td>
<td>0.15</td>
</tr>
<tr>
<td>80</td>
<td>39.0</td>
<td>0.25</td>
</tr>
<tr>
<td>70</td>
<td>43.6</td>
<td>0.38</td>
</tr>
<tr>
<td>60</td>
<td>48.6</td>
<td>0.54</td>
</tr>
<tr>
<td>50</td>
<td>53.5</td>
<td>0.83</td>
</tr>
<tr>
<td>40</td>
<td>58.1</td>
<td>1.20</td>
</tr>
<tr>
<td>30</td>
<td>62.7</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Fig. 2. Logarithmic relationships between initial rates and initial concentrations of \((\ominus)\) \( p \)-Benzoquinone with excess barbituric acid and of \((\Delta)\) Barbituric acid with excess \( p \)-benzoquinone in 50% MeOH-H\(_2\)O mixture at 30°C.
3.2. Variation of rate constant with solvent composition

In Table 3, the second order rate constant \( k_2 \) for the reaction of \( p \)-benzoquinone and barbituric acid in methyl alcohol–water media of different compositions at 30°C, decreased with increasing organic solvent component. The variation of log \( k_2 \) with the reciprocal of the bulk dielectric constant \( D \) of the mixed solvents [16] is linear as shown in Fig. 3. This indicates that the transition state is more polar than the reacting species [17]. Increasing the water content in the solvent mixtures increases the rate of the reaction by stabilizing the transition state.

3.3. Determination of the quinones tested

The nature, stability, rate and the intensity of the color developed by the reaction of the quinones tested with excess barbituric acid depended on the type of quinone used and the composition of the solvent.

3.3.1. Effect of time and temperature

The rate of color development due to reaction of the quinones tested with excess barbituric acid in 50% methyl alcohol–water mixture had reached a maximum intensity after 5 and 15 min at 30°C for \( p \)-benzoquinone and \( p \)-chloranil respectively and after 1 h at 50°C for 1,4-naphthoquinone, as shown in Tables 4 and 5.

3.3.2. Effect of variation of solvent composition

The change in color development for different compositions of methyl alcohol–water mixture due to reaction of quinones tested with excess barbituric acid at 30°C was studied spectrophotometrically. Fig. 4 shows that the absorption reaches a maximum as the percentage of water increases up to between 50 and 60%.

In summary \( p \)-benzoquinone, 1,4-naphthoquinone and \( p \)-chloranil have been shown to be quantifiable with a new and simple analytical method. This method offers the advantages of: (a) reagent availability and stability; (b) reasonable selectivity in the presence of other quinones, such as 9,10-anthraquinone and phenanthrenequinone, which were found not to interfere; (c) rapidity; and (d) high sensitivity compared to other recently proposed spectrophotometric methods [12,13] which use buffered solutions.

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Time /min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>( p )-Benzoquinone</td>
<td>0.14</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>–</td>
</tr>
<tr>
<td>( p )-Chloranil</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 5
Effect of temperature on absorption of the product of reaction
of quinones tested with excess barbituric acid in 50% methyl
alcohol–water mixture

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>p-Benzquinone</td>
<td>0.10</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>0.16</td>
</tr>
<tr>
<td>p-Chloranil</td>
<td>0.20</td>
</tr>
</tbody>
</table>

3.4. Determination of barbituric acid

A new spectrophotometric method is applied to
the estimation of barbituric acid with p-benzo-
quione in 50% methyl alcohol–water. This
method is simple, more sensitive and the color
produced is stable for longer period. Moreover,
by this method, barbituric acid can be estimated
in the presence of various of its derivatives, which
do not produce color with p-benzoquinone. A
calibration graph for barbituric acid was obtained
by transferring 0.1–1.7 mL aliquots of (2.5 x 10–3 M) solution in methyl alcohol to test tubes
followed by addition of 1.0 mL of (0.05 M)
methanolic solution of p-benzoquinone and the
appropriate volume of water to attain 50% methyl
alcohol–water mixture. The mixtures were heated
at 50°C for 30 min in water bath. The mixtures
then cooled, diluted to 10 mL with 50% methyl
alcohol–water mixture and the absorption at 500
nm measured. Beer’s law is obeyed in the concen-
tration range of 0.025–0.345 mM of barbituric
acid.

This method can be applied for checking the
purity of barbiturate drugs which may contain
small amounts of barbituric acid as an impurity,
while not containing any other organic comp-
ounds (such as amines and amino acids) which
produce color with p-benzoquinones.

3.5. Reaction mechanism

No reference was found to the reaction of
quinones with barbituric acid, which is an active
methylene compound. In previous work, however,
non-hydroxylated quinones, having a free
quinonoid position, have been detected by their
reaction with active methylene compounds [18]
such as acetoacetic ester, malononitrile and ni-
has shown that the anion of the reactive
methylene group undergoes Michael addition and
subsequent oxidation produces a new quinone. In
the present work, measurement of the absorbance
of various molar ratios of the quinones for reac-
tion with barbituric acid in 50% methyl alcohol–
water mixtures shows that the maximum
absorption appears at the molar ratio of 1:1 for
the quinones tested. Elemental analysis and in-
frared spectra of the reaction product between
barbituric acid and 1,4-naphthoquinone, for ex-
ample, are very close to those expected for a
mono-adduct (I), as shown in Scheme 1.
According to the kinetic data, a similar mechanism to that proposed by Jeffrey [20] can be outlined as in Scheme 2.

This mechanism has been supported by the following indirect evidence:
(1) There is no reaction between the quinones tested and either 5,5-diethylbarbituric acid or phenobarbitone due to the absence of the active methylene group;
(2) There is no interaction between barbituric acid and the unfree quinonoid position compounds such as 9,10-anthraquinone or phenanthrene-quinone.

References