Of the many methods that have been proposed for the direct chemical determination of vitamin C in plant and animal tissues, those based upon the reduction of 2,6-dichlorophenol indophenol have been adopted far more widely than others. The original indophenol procedure proposed by Tillmans and associates (1) a short time before the vitamin was isolated and identified (2) was soon modified in varying degrees in a number of laboratories (3–6). The indophenol method depends upon the fact that vitamin C is the major or only natural tissue component which reduces the dye rapidly in an acid solution (e.g., pH 2 to 4).

The present paper deals primarily with the value of having metaphosphoric acid in the acetic acid or trichloroacetic acid used for extracting and titrating the vitamin. In general we have found the procedure outlined by Bessey and King (5) more satisfactory than other methods which have been proposed for tissue analysis, and many other laboratories appear to have had essentially the same experience. The first suggestion of using metaphosphoric acid which came to our attention was in the paper by Fujita and Iwatake (7).

**EXPERIMENTAL**

*Atmospheric Oxidation of Vitamin*

It seems reasonably evident that nearly all tissues containing vitamin C also contain one or more enzymes which catalyze its aerobic oxidation to a marked degree as soon as the cellular tissue

* Contribution No. 321 from the Department of Chemistry, University of Pittsburgh.
Determinative of Vitamin C

is crushed or severely injured (8-11); hence this factor is of extreme
importance in tissue analysis. We have found that it is generally
essential to immerse the tissue in a strong acid before it is chopped
fine or ground for extraction. Fluids such as urine, milk, and
vegetable or fruit juices should be collected directly into an acid,
preferably metaphosphoric acid with acetic or trichloroacetic
acid, in such quantity that the final concentration is about 2 per
cent of the former and 4 to 8 per cent of the latter. In working
with animal tissues, such as tumors (12), we have found it advan-
tageous to freeze the tissue in dry ice until it is ready for extraction.
It is then pulverized in a glass mortar, covered with the mixed
acid (before thawing), then ground, and extracted as usual.

Minute amounts of dissolved copper also catalyze atmospheric
oxidation (13), and since the ordinary distilled water, reasonably
clean laboratory apparatus, and many c.p. reagents contain sig-
ificant quantities of copper, the hazard from this factor is rela-
tively great. It is reasonably certain that reports of finding
appreciable amounts of the vitamin in the reversibly oxidized
state in natural fresh tissues have been in error because of second-
ary oxidation. The apparent rôle of glutathione in maintaining
the vitamin in a reduced state \textit{in vitro} and \textit{in vivo}, and the second-
ary irreversible change (\textit{in vitro}) of dehydroascorbic acid in neutral
solution are items of particular importance in relation to this view
(13-15).

The cellular structures in some tissues are disrupted more rapidly
by trichloroacetic acid (4 to 8 per cent) than by metaphosphoric or
acetic acids, and the former also exerts a better deproteinizing
action. However, in the presence of trichloroacetic acid alone
the vitamin is oxidized at an appreciable rate and may thus intro-
duce a serious error, particularly in delayed titrations. Occa-
sional lots of the acid are very bad in this respect, and should be
discarded. The observed oxidative effect was not related to the
hypochlorite or copper content of the acid.\(^1\) The metaphosphoric
acid (1 to 5 per cent) exerts a protective effect against both
atmospheric and trichloroacetic acid oxidation.

In preliminary work it was found that the addition of 2 per cent

\(^1\) The oxidation is accompanied by the liberation of \(\text{Cl}^-\), analogous to
the observed oxidation of \(\text{Cu}^+ \rightarrow \text{Cu}^{++}\), reported by Neuberg and Kobel
(10).
TABLE I

*Stabilizing Effect of Metaphosphoric Acid upon Vitamin C in Solution*

Each solution containing 0.4 mg. of vitamin C per 5 cc. was exposed to the air at room temperature in a 50 cc. Erlenmeyer flask.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Per cent destroyed after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 hr.</td>
</tr>
<tr>
<td>Acetic acid, 8%</td>
<td>0</td>
</tr>
<tr>
<td>&quot; 8% + 0.01 mg. Cu++</td>
<td>8</td>
</tr>
<tr>
<td>+ HPO₃, 2%</td>
<td>0</td>
</tr>
<tr>
<td>HPO₃, 2%</td>
<td>0</td>
</tr>
<tr>
<td>&quot; 2% + 0.01 mg. Cu++</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄COOH, 8%</td>
<td>46</td>
</tr>
<tr>
<td>Sample A</td>
<td>95</td>
</tr>
<tr>
<td>&quot; B</td>
<td>0</td>
</tr>
<tr>
<td>&quot; C</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄COOH (B), 8%, + HPO₃, 2%</td>
<td>75</td>
</tr>
<tr>
<td>&quot; 8% + 2%</td>
<td>23</td>
</tr>
<tr>
<td>+ 0.01 mg. Cu++</td>
<td></td>
</tr>
<tr>
<td>Water, distilled supply</td>
<td>0</td>
</tr>
<tr>
<td>&quot; tap</td>
<td>0</td>
</tr>
<tr>
<td>&quot; redistilled from quartz</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE II

*Influence of Extractants on Vitamin C Titration Values (Mg. per Gm.)*

<table>
<thead>
<tr>
<th>Animal and plant tissues</th>
<th>Extractant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPO₃, 2 per cent</td>
</tr>
<tr>
<td>Beef adrenals</td>
<td>1.07</td>
</tr>
<tr>
<td>&quot; spleen</td>
<td>0.44</td>
</tr>
<tr>
<td>&quot; pancreas</td>
<td>0.11</td>
</tr>
<tr>
<td>Green peppers</td>
<td>1.72</td>
</tr>
<tr>
<td>Potatoes</td>
<td>0.10</td>
</tr>
<tr>
<td>Turnips</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Determination of Vitamin C

metaphosphoric acid to standard solutions of vitamin C in specially redistilled water, ordinary distilled water, tap water, acetic acid solution, and trichloroacetic acid solution, delayed oxidation when the flasks were exposed to air at room temperature. This was also true when small amounts of copper salts were added, as indicated in Table I.

The results given in Table II indicate clearly the advantage of using 2 per cent metaphosphoric acid with 8 per cent trichloroacetic acid or acetic acid when grinding tissue for extraction and titration of the vitamin. Each value given in Tables I and II represents an average of six or more determinations which were in close agreement.

SUMMARY

The titration procedure of Bessey and King for the determination of vitamin C has been modified to include the presence of 2 per cent metaphosphoric acid with acetic acid or trichloroacetic acid during extraction and titration. The modified procedure is advantageous for work with both plant and animal tissues.

Metaphosphoric acid in approximately 2 per cent concentration, as suggested by Fujita and Iwatake, serves to protect vitamin C in solution against atmospheric oxidation, even in the presence of added copper, and also exerts protective action against oxidation in the presence of trichloroacetic acid. The rate of reaction with 2,6-dichlorophenol indophenol is not appreciably affected by the presence of metaphosphoric acid.

BIBLIOGRAPHY


