MICROGRAM ANALYSIS: A SOLID-LIQUID EXTRACTOR AND ITS APPLICATION TO EXTRACTION OF SUGARS FROM PLANT MATERIALS*

BY HERBERT STERN† AND PAUL L. KIRK
(From the Division of Biochemistry, University of California Medical School, Berkeley)

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In adapting methods of continuous solid-liquid extraction to the microgram range, the problem is essentially that of efficiently refluxing 75 to 200 μl of solvent. For this at least two conditions are necessary: The bulk of extracting fluid at any given moment should be in the liquid state, and the condensation should be so arranged to deliver to the extraction cup or thimble sufficiently small droplets without loss to other parts of the vessel. The first of these requirements would hardly be satisfied by most of the micro extractors described by Schneider (1) because of their relatively large size; the second of these becomes troublesome when an attempt is made to scale down the size without modification of design. In general, any type of hollow tube condenser, air- or water-cooled, appears undesirable, since the downward flow of condensate is too frequently obstructed by droplets trapped in the narrow tube by capillary forces. The cold finger type of condenser certainly appears preferable, but even so, the simple expedient of inserting one into a small test-tube is rendered ineffective by the trapping of liquid at various points between walls of condenser and test-tube. We found a large condensing surface within a small volume and with a steep temperature gradient between vessel wall and condenser to be a modification necessary to the successful functioning of a drop scale extractor. Based upon this principle, the extractor described here was designed. With it 75 to 300 μl of volatile solvent (alcohol or ether) could be refluxed for several days without appreciable loss in the volume of extraction medium.

In so far as the mechanics of the extractor were concerned, these could be observed and judged by using a variety of solvents. It was desired, however, in view of our interest in the metabolism of pollen mother-cells, to test the apparatus for extraction of microgram quantities of sugars. Also, in order to complete the procedure, we devised and tested means of filtration and evaporation of very small volumes.

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† Holder of a Royal Society of Canada Fellowship, 1947–48. Present address, Medical School, Witwatersrand University, Johannesburg, South Africa.

1 μl = 10⁻⁶ liter = 0.001 ml.
**Description of Extractor**

The essential features of the apparatus are illustrated in Fig. 1. In its construction it is desirable to make the supporting pegs of minimum diameter. (We achieved this by using a micro torch, fusing a droplet of glass at the desired point, heating the glass locally, and quickly jabbing it with a dissection needle.) It is also advisable to keep the total capacity of the reservoir bulb (including neck) to 400 \( \lambda \) or less, if the volume of solvent to be used is of the order of 200 \( \lambda \). Cones of platinum or glass are equally functional; the tail attachment is necessary for a smooth and directed flow of solvent. The diameter of the condenser bulb should be as large as the neck of the extractor vessel will allow. The height of the air outlet we have not found to be critical.

**Operation of Extractor**

*Heating—*Our experience indicates that the use of nichrome resistance wire attached to a variable voltage transformer is by far the most favorable method of heating. Control of temperature is sensitive and easy. The
current can be adjusted to boil liquid without spattering (we have tried water, alcohol, and ether with equal success) and to any desired rate of droplet condensation. In fact, once the proper voltage is found, adjustments for subsequent extractions are unnecessary. With respect to the nichrome wrapping, it is important that only one or two coils are maintained around the lower bulb, the remainder being distributed over the upper portion of the extraction vessel.

**Extraction Cup**—The arrangement here is arbitrary. We inserted a small cotton plug at the base which served not only to support the tissue but also to control the level of liquid in the cup. By adjusting both plugging and heating it is possible to maintain the liquid in the cup at any desired level. Small filter cones were also tried and they functioned equally well. In both cases, the tail of the extraction cup should be so positioned that the droplets fall directly into the reservoir without hitting the walls. Adjustment, if necessary, is done by gentle tapping of the apparatus.

**Evaporation of Solvent**—This is achieved by halting the water circulation, removing the extraction cup, and attaching the air outlet to a vacuum line. The applied voltage is the same as that used for refluxing. Evaporation in this way is smooth and quick, about 5 minutes being required for total removal of 80 per cent ethanol.

**Extraction of Sugars from Onion Root Tip**

To provide experimental material, an onion (*Allium cepa*) was suspended over a beaker of aerated water kept at room temperature and the roots thus formed used in the experiments here described. (Anthers of *Trillium*, for which the research was intended, were unavailable at the time.) Since a physiological study was not being conducted, no attempt was made to control growth conditions. The roots were sliced and their fresh weight determined by a quartz helix balance (2). The portions used were all of the order of 0.3 to 3 mg. As suggested by Hassid (3), 80 per cent ethanol was used for extraction medium and the material refluxed for periods varying from 5 to 24 hours. Maceration of the root appeared unnecessary, since the yields after 6 hours were no less than those obtained after 20 hours of extraction. Following extraction, the alcohol was evaporated down to about 10 λ and then transferred, with several washings, to a micro test-tube. It is possible to add reagents directly to the extraction bulb, but this is less convenient when centrifugation is desired. The contents of the test-tube, about 150 λ in volume, were again evaporated to 10 λ.

Evaporation of solvent from a narrow tube is more difficult than from a bulb with a relatively large surface. It was satisfactorily accomplished, however, by using the arrangement indicated in Fig. 2. The essential point is the use of the nichrome wire to heat the chamber but not the tube.
If a low voltage is first applied and then increased stepwise, evaporation proceeds smoothly and is complete in about 30 minutes. The required voltages need only be established once; evaporations may then be run with little care.

To the 10 \( \lambda \) of extract in the tube were added 5 \( \lambda \) of saturated neutral lead acetate, and the sides of the vessel carefully rinsed with an additional 5 to 10 \( \lambda \) of water. 10 \( \lambda \) of saturated disodium phosphate were measured into the mixture, the contents well mixed, and 5 or 10 minutes later centrifuged with the air centrifuge.\(^2\)

![Figure 2](image1.png)

**Fig. 2.** Evaporation assembly. \( a \), nichrome wire leads to variable voltage transformer; \( b \), outlet to vacuum.

![Figure 3](image2.png)

**Fig. 3.** Microgram filtration assembly. \( a \), vacuum outlet; \( b \), supporting glass wool; \( c \), calibrated micro tube; \( d \), ground glass top of capillary tube on which filter paper rests.

The type of filter we found most suitable is shown in Fig. 3. It is, in fact, generally useful when samples in the neighborhood of 50 \( \lambda \) are to be filtered and a quantitative recovery of filtrate is desired. The apparatus is easily made with a short piece of capillary tubing, 1 mm. bore, by enlarging the bore at one end and grinding that end flat. The other end is drawn out and bent slightly at the tip to provide a small point of contact between it and the wall of the calibrated micro tube. It is also advisable to paraffin the lower portion. Suitable filtering disks were obtained by means of a paper punch. In operation, the filter paper was wetted and the suction turned on to assure adhesion of the disk to the ground glass surface. The suction was then stopped and a calibrated micro test-tube

\(^2\) Obtainable from the Microchemical Specialties Company, Berkeley, California.
A capillary pipette of rather large bore was used to take up successive portions of the mixture which was applied dropwise to the filter disk. The vacuum was so regulated as to provide a smooth flow of filtrate down the side of the tube and no accumulation of liquid on the filter paper. When means of adequate centrifugation are not available, the mixture of precipitate and solution can be filtered directly, but to avoid plugging of the filter, talc is first applied as described by Hassid (3).

The efficiency of this method of clearing was tested with 4 μ samples of glucose. 10 λ of glucose solution containing 4 μ of glucose were delivered to a sugar tube, and the reagents added as described above. Controls were simultaneously run in all cases. The results are shown in Table I, where the volume of ceric sulfate required for the filtrate as a percentage of that required for the control is given.

### Table I

**Effect of Clearing and Filtration on Glucose Recovery**

Recovery in individual samples expressed as percentage of controls.

<table>
<thead>
<tr>
<th>Samples centrifuged and filtered</th>
<th>Samples filtered without centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.5</td>
<td>99.5</td>
</tr>
<tr>
<td>100.1</td>
<td>100.5</td>
</tr>
<tr>
<td>101.1</td>
<td>100.4</td>
</tr>
<tr>
<td></td>
<td>98.8</td>
</tr>
</tbody>
</table>

In the case of plant extracts, the filtrate was made up to 200 λ and 40 λ aliquots used for determination of total sugars and reducing sugars. The size of aliquot is, of course, arbitrary and depends largely upon the total sugar content of the extract. Since even 0.28 mg. of root yielded about 8 μ of sugar, it was unnecessary in our case to increase the size of aliquot. A very important point at this stage of the procedure is the mixing of the filtrate once made up to volume. Apparently, inversion and twirling of the tube are inadequate, for quite often if treated this way, the aliquots showed a gradation in concentration, the first one removed always being the lowest. The use of a 200 to 250 λ pipette, paraffined on the outside, obviated the difficulty. By successive drawing up and releasing of the solution at different levels in the tube, thorough mixing was achieved.

Again to test for reliability of the method, the recovery of five samples of sucrose was studied (Table II). The variability of the sugar content between different roots and, as would appear from our results, between different portions of the same root precluded the adoption of any particular concentration as a standard. Instead, both these methods were tried: The extract was divided equally, and to one-half, 4 μ of sucrose were added,
or, after 6 hours extraction, the extract was similarly divided, one-half was returned to the extraction bulb, 4 y of sucrose added to the extraction cup, and the solution again extracted for a few hours. Both methods yielded similar recoveries.

In view of the successful recovery, some comparisons were made between the sugar content of different roots and between two different portions of the root. The results are listed in Table III. The values are all expressed in terms of sucrose equivalents.

### Table II

**Recovery of 4 y of Sucrose in Extracts of Various Sugar Concentrations**

<table>
<thead>
<tr>
<th>Total sugar content of sample</th>
<th>Recovery per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.8</td>
<td>104</td>
</tr>
<tr>
<td>78.6</td>
<td>98</td>
</tr>
<tr>
<td>25.0</td>
<td>100.2</td>
</tr>
<tr>
<td>8.3</td>
<td>101.1</td>
</tr>
<tr>
<td>7.6</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table III

**Sugar Content of Onion Root**

<table>
<thead>
<tr>
<th>Length of root</th>
<th>Distance of section from tip</th>
<th>Weight of sample</th>
<th>Total sugar per mg. fresh weight</th>
<th>Reducing sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 cm.</td>
<td>Tip</td>
<td>3.4</td>
<td>23.5</td>
<td>6.5</td>
</tr>
<tr>
<td>3 cm.</td>
<td>0.5</td>
<td>1.1</td>
<td>17.4</td>
<td>2.9</td>
</tr>
<tr>
<td>3 cm.</td>
<td>Tip</td>
<td>0.28</td>
<td>28.0</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.90</td>
<td>26.0</td>
<td>6.9</td>
</tr>
<tr>
<td>3 cm.</td>
<td>Tip</td>
<td>1.73</td>
<td>24.5</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.50</td>
<td>28.1</td>
<td>4.6</td>
</tr>
<tr>
<td>1.6 cm.</td>
<td>Tip</td>
<td>0.70</td>
<td>21.6</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.89</td>
<td>19.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

From these results it is apparent that a great deal of variability exists with respect to sugar content. Some of the differences might be real, the higher proportion of reducing sugars at the tip, for example. Others may well be due to variability in water content of the tissues. It is clear, however, that, in so far as the method of extraction is concerned, there are no outstanding differences in recoveries from small or large root sections. This latter point, of course, is our chief interest here.

**SUMMARY**

A microgram extractor, filter, and evaporator are described, as well as the technique necessary in applying this equipment to the analysis of...
microgram quantities of sucrose or other non-reducing sugars. The method was tested in the analysis of short lengths of root tips.

BIBLIOGRAPHY

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Herbert Stern and Paul L. Kirk


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