

THE CIRCULAR CHROMATOGRAPHIC METHOD ACCORDING TO E. E. FEEBEEPRACTICAL INSTRUCTIONSExtraction of Samples:

In the Biochemical Research Laboratory, Spring Valley, New York, many different methods of extraction and degrees of concentration of samples have been tried out. In the following, we report only the standards which have given us the most satisfactory results and which can be used for comparison with our test materials and for our way of interpretation. It must be realized that any change of extraction or of concentration changes the observable pattern, so that chromatograms deriving from differences in the preparatory steps cannot be compared. Only those which have been prepared exactly alike can be compared. For all other cases, new standards of interpretation must be developed. Also concentrations and extraction time may need variations occasionally.

1. Concentration of nitrate of silver ( $\text{AgNO}_3$ ) solution made from crystals of certified reagent of purest, analytical quality F. W. 169.888: 0.5% in pure aqua dest. in all cases. This solution needs to be prepared fresh at least every other week and should be kept in a dark, glass stoppered bottle. As soon as a discoloration or precipitation shows up, it can no longer be used. This solution is used for sensitizing the filter paper disk.

2. Concentration of sodium hydroxide solution ( $\text{NaOH}$ ) made from sodium hydroxide electrolytic pellets, purest quality, F. W. 39.999, by dissolving exactly 1 gr. in 1000 ml. pure aqua dest. to make an 0.1% solution. This solution is used for extraction of the sample in all cases, unless otherwise specified below. It should be kept in a well stoppered flask in order to avoid evaporation of water during storage. In case it shows discoloration (from stopper, for instance) or incrustation on the neck of the bottle or any sediment, discard it.

3. Filter Paper: Round disks of genuine Whatman Filter Paper #1 from a reliable company, 15 cm. diameter, average ash per circle 0.0009 gr. or less. Special chromatographic filter paper disks may be used too if of the same diameter. Careful handling of these disks by touching only the edges, never the surface, is necessary in order to avoid fingerprints. Store in dry air and always have boxes covered. The disks should be sensitized with an 0.5%  $\text{AgNO}_3$  solution on the day of the test. For the actual test, these prepared disks should not be older than 4 hours and should be perfectly air-dry before using the addenda, that is, the test solutions.

Preparation of grains, such as wheat, rye, oats or barley: Grains are ground up, as received, in a laboratory grinder, such as Wiley Multi-Cut Mill (for materials with a low oil content). This mill contains shearing plates and shreds, rather than mills like a flour mill. It is important that the material pass through the mill in the shortest possible time, without heating or steaming. The sample should be processed immediately after grinding. For small grain, we use the finest setting of the mill, for corn we pre-grind coarsely and a second time with the finest setting. Ground-up samples should not be stored for any length of time. We usually use 12. gr. of sample in order to have enough material on hand.

For small seeds: grind cautiously (not to heat up) in nut mill; of the ground material transfer 1 gram into Erlenmeyer flask, add 50 cc of 0.1% NaOH soln; mix by twirling at the start, after 15' and again after 30'. Whole extraction time: 4 hours.

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Use 2.25 gr. of ground up sample, put in an Erlenmeyer flask holding 125 ml., add 50 ml. of the 0.1% sodium hydroxide solution (see #2), mix thoroughly by twirling the flask, repeat after 15 minutes and then let stand for 14 hours. Cover the Erlenmeyer with an inverted glass beaker. Usually the extraction is started in the late afternoon to be ready the next morning. Decant carefully the supernatant fluid into a glass beaker of about 50 ml. capacity and measure 5 ml. with a small measuring cylinder for each flat bottom, capsule form Coors porcelain crucible (capacity 10 ml., diameter 45 mm., height 12 mm.).

This is our standard method for comparison.

We also run a test with 1.5 gr. of ground up sample in 50 ml. of the 0.1% NaOH solution, proceeding otherwise as described for the 2.25 gr. sample. This method gives additional information if needed. It does not always show the drastic differences as the other.

Preparation of flour and dough: Flour and dough are used as received from the outside or as freshly prepared in the laboratory, as the case may be. Again, we use 2.25 gr. and 1.5 gr. samples and proceed exactly as with the above-mentioned method.

Preparation of bread of all kinds: The measured amount of bread is mortared a few minutes in a porcelain mortar with a small amount of the NaOH 0.1% solution to make a homogeneous paste. 2.5 gr. of bread are used for our standard determination and 1.5 gr. of bread for supplementary information. Proceed as described above, except that the extraction time in this case is 4 hours.

Preparation of fresh green leaves (all kinds), vegetables, fruit, nuts: The incoming material should be as fresh as possible. It is first cut as finely as possible with clean scissors (not rusty) and then mortared. Use 2.5 gr. of the finely cut material to 50 ml. of the 0.1% NaOH solution and continue as previously described. The time interval between cutting, mortaring and adding to the extracting solution should be kept as short as possible. The extraction time for all these products is 4 hours. Incoming material can be kept in a refrigerator at about 40°F. Special storage and research on keeping quality may demand a different handling of the source material, but the method of extraction, etc., remains the same.

Preparation of roots, tubers and bulbs: These are finely grated prior to mortaring; then one proceeds as for leaves, vegetables, etc. Use 2.5 gr. of material to 50 ml. NaOH, 0.1%. Extraction time 4 hours.

Preparation of green herbs: Fresh green material is finely cut, mortared and processed as for fresh green leaves; that is, no tea or infusion is brewed. Our standard is 2.5 gr.; because of grade variations in strength, 1.5 gr. may be needed for supplementary information.

Dried herbs and teas: Two paths of investigation are open: (a) to proceed as with any other dried or dehydrated material (no recommendation can be given at this moment); (b) as tea; in that case, an

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infusion of the dry herb or tea is made by putting 2.5 gr. in 50 ml. aqua dest. and bringing it up to boiling point without losing fragrance; then add immediately 50 ml. NaOH, 0.1%, let stand for 2 hours and proceed as usual. A cold extraction might be used too.

In the case of very strong teas (black) containing lots of extractives, even a direct chromatogram of the tea alone could be tried. In that case, 5 gr. may be used in 100 ml. water.

Preparation of juices, fruit juices, soft drinks, pressed extracts: The juices are not filtered but used as they are received, using a juicer. 5 ml. are added to 5 ml. of the 0.1% solution. From here on, proceed as usual, but extraction time is only 1 hour.

Enzyme preparations: Of the pure enzyme preparations in dry powder form, 0.1 gr. is used. 10 ml. of 0.1% NaOH solution are used for all enzymes which are active in alkaline media, while 10 ml. of 1.0% HCl solution are used for enzymes, active in acid solution. Extraction time 1 hour.

Yeast preparations: 0.1 gr. of the dried yeast powder or yeast cake is extracted in 10 ml. of 0.1% NaOH solution. Extraction time 1 hour.

Vitamin preparations, single, pure or in mixture (i.e., complex formula): The concentration depends somewhat on the strength of the preparation. To begin with, we use 0.1 gr. in 10 ml. of 0.1% NaOH solution. Extraction time 1 hour. It might be necessary to modify this concentration. The spread is between 0.5 gr. to 0.01 gr.; for pure crystalline vitamins, as little as 0.001 gr. may be sufficient. If chromatograms get too dark, one can reduce either the amount of substance or increase the ml. of the NaOH solution. Also, if different vitamin preparations are to be compared, it is necessary to calculate the actual formula to have the same amount or strength of each vitamin, especially if comparison with pure vitamins is desired.

Dried foods like macaroni, egg noodles, etc.: Use 2.5 gr. in 50 ml. NaOH, 0.1%. Extraction time 4 hours.

Sugars, honey, molasses, maple syrup: In general, 2.5 gr. in 50 ml. 0.1% NaOH solution are used. Extraction time 4 hours.

Milk: Fluid milk 2.5 gr. in 50 ml. 0.1% NaOH solution. Extraction time 1 hour.

Dried, dehydrated milk powder: 0.1 gr. in 10 ml. 0.1% NaOH solution. Extraction time 1 hour.

Fats and oils: Do not use the above method; a special method has been worked out. Instructions will follow.

Soils and compost: 5 gr. of the air-dried sample are used in 50 ml. 1.0% NaOH solution. Extraction time 6 hours. Mix well; shake. Shake again after 15 minutes and once more after 1 hour; let stand. All soils are used air-dried, visible roots and pebbles removed; then they are slightly mortared before use. Compost is handled the same way.